“BIOGAS GENERATION POTENTIAL OF COCONUT COPRA IN THE ANAEROBIC DIGESTION PROCESS”

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Abstract

Pacific Island Countries (PICs) currently rely heavily on imported, expensive and unsustainable fossil fuels as their primary source for energy production. Establishing an alternative energy source from local resources would therefore have considerable benefits for many Island communities. Coconut copra is high in biodegradable organic carbon and is found growing abundantly in PICs. One alternative use for this local resource would potentially be the conversion of the coconut copra into a valuable and useful energy source through biological processes, such as anaerobic digestion.

The purpose of this research was to investigate the biogas generation potential of coconut copra as a carbon source using anaerobic digestion processes. Both batch and continuously stirred reactors (CSTRs) in addition to the environmental and operating variables that affect the production of biogas were investigated in order to optimize methane production and increase the overall conversion efficiencies of organic matter to methane.

The results suggest that coconut copra is amenable to anaerobic digestion due to the high theoretical methane yields from the substrate's high lipid content. However, the optimal organic loading rate (OLR) was limited to within a narrow range of 3.6-6 g VS (2.4 - 4 g VS/L Reactor) for the batch reactors and a maximum of 0.420 L CH_4/ g VS was achieved at an OLR of 3.6 g VS. OLRs exceeding 15 g VS resulted in low pH values and negligible methane production due to substrate overloading. High average methane yields of 0.708 L CH_4/ g VS·day were also successfully achieved for the CSTRs and increased mixing was observed to have an improved effect on methane production. However, the addition of

nitrogen and phosphorus supplements failed to increase biological activity and ultimately resulted in the accumulation of ammonia to concentrations toxic to methanogenic bacteria. The failure of an accelerated CSTR start-up procedure also reinforced the requirement for a gradual and steady acclimated period for anaerobic digestion of this particular substrate.

Acknowledgements

"Every worthwhile accomplishment, big or little, has its stages of drudgery and triumph; a beginning, a struggle and a victory." (Mahatma Ghandi)

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1 Literature review

1.1 Fuels in the Pacific

"*We have become far too dependent on imports of fossil fuels during the past half century. Looking to the future, we have committed to a reduction and eventual elimination of fossil fuel for energy production" (WorldBank 2004)*

Currently fuel prices in Pacific Island Countries (PICs) are among the highest in the world with some of the smaller island communities paying 70-140% more than the wholesale price for petroleum than some developed nations (MacKenzie 2009). Most PICs do not have their own indigenous petroleum resources and currently hydroelectric generation projects in PICs are very few and are limited to Fiji and Vanuatu (Jayaraman and Choong 2009). Instead, many Pacific Islands rely heavily on imported fossils fuels to generate electricity to the extent where they are typically the single largest import item to these countries and exceed the total domestic commodity export by up to 500 % (Jafar 2000). This reliance on imported energy also makes the economies of PICs vulnerable to fluctuations in global petroleum and diesel prices. In addition, local national currencies have devalued significantly in recent years so that in local currency terms, the cost of imported fossil fuels has further increased (Etherington 2005).

Ensuring a consistent energy supply is compounded because the capacity for bulk storage is limited and many of the islands are located thousands of kilometres from the major petroleum markets. Often island communities have insufficient port infrastructure to allow for larger shipments of fuel therefore distribution of fuel to remote areas is restricted to canoes carrying small quantities (Etherington 2005). The combination of import duties on arrival, the cost of repackaging and the addition of a sales margin at each transfer step, results in a significant

fuel increase for rural PIC communities. Fuel costs on some outer islands can be as much as 200-400% higher than those on the main islands (MacKenzie 2009).The increasing dependency on imported fossil fuel is also a major constraint to environmentally sustainable, socio-economic development for many of the 22 political entities that make up the Pacific Island region. The limited availability and high cost of energy production in rural areas has only added to the increasing trend of urban migration (Yu and Tapling 1997). The delicate island ecosystems of the Pacific region are also extremely vulnerable to environmental damage, habitat loss and pollution resulting from conventional energy sources.

In this respect, there is a large economic as well as environmental incentive to reduce dependency on imported fossil fuels by obtaining energy sourced from local resources. To date renewable energy systems have not made significant contributions to the energy supply of many of the Pacific Island nations due to a number of limitations (Yu and Tapling 1997). These include the challenge of engineering technologies and appropriate systems that are able to use local materials at a cost that local communities can afford (Chen et al. 2007).

1.2 Coconut Copra

The coconut palm (*Cocos nucifera L.*) grows abundantly in many countries including Indonesia, India, Philippines and the Pacific Islands with world production at approximately 10 million tonnes a year (Cloin 2005a). The coconut palm is considered a versatile plant with a variety of uses and every part of it can be incorporated into a number of industries from food supplies, drink, and shelter to raw materials. One advantage of the coconut palm is that it produces fruit continuously throughout the year with only minor seasonal variation.

In addition, the lifespan of the coconut palm can be up to 70 years with each palm producing up to approximately 80 seeds or kernels per annum (Banzon 1984). The coconut fruit or kernel is a valuable source of coconut oil and copra, while the husk, shell and petiole (leaf stalk) are usable biomass.

Coconut palms are ideally suited to the humid, tropical costal climates of PICs and established plantations currently cover thousands of hectares of land in these regions. The local island communities often refer to the coconut palm as the "the tree of life" because of its various uses and its ability to withstand extreme weather conditions (Banzon 1984).

The mature coconut fruit (drupe) consists of three layers; an exocarp, a fibrous mesocarp and the endocarp or shell (Figure 1.1). The endosperm is the edible portion that develops within the endocarp and possesses two distinct raw materials, wet meat or copra and coconut water (Figure 1.2).

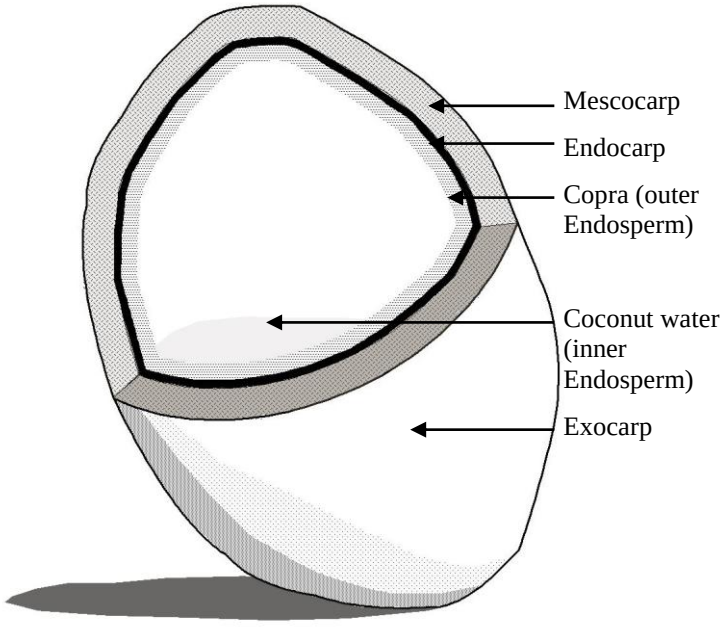

Figure 1.1: Mature coconut fruit diagram

Figure 1.2 : Endocarp with coconut water removed and copra visible

When the wet copra is dried for commercial and domestic use, the moisture content of the copra is reduced from approximately 50-55% to less than 5% (Santoso et al. 1996). Dried coconut copra has a fat content of approximately 55-65%, 23% carbohydrates and 7% protein depending on the age of the coconut, growing conditions and variety (Santoso et al. 1996). Approximately 90% of the total fat consists of medium and long chain fatty acids (LCFAs) with the most predominant being lauric acid ($C_{12}H_{24}O_2$) followed by myrsitic ($C_{14}H_{28}O_2$) and palmitic acids ($C_{16}H_{24}O_2$).

Although coconut palms are one of the principal crops of the Pacific Islands the decreasing world market prices and high transport costs has resulted in a decline over the last 10 years of the export of coconut copra and oil (Cloin 2005a). In the past, the export market for coconut products has been economically viable, however, the current international market value for coconut copra and coconut oil has become highly volatile and the quoted price can double or halve within a given year (Etherington 2005). The World Bank also forecasts that world coconut copra and oil prices will continue to remain low over the coming decade as they are out competed by other plant sources such as palm, corn and canola oils (Cloin 2005a).Extreme fluctuations in the market prices together with increasing transportation costs has therefore resulted in an overall decrease in coconut harvesting and export, leaving many crops unharvested in plantations since exporting to overseas markets has become uneconomical (Figure 1.3)

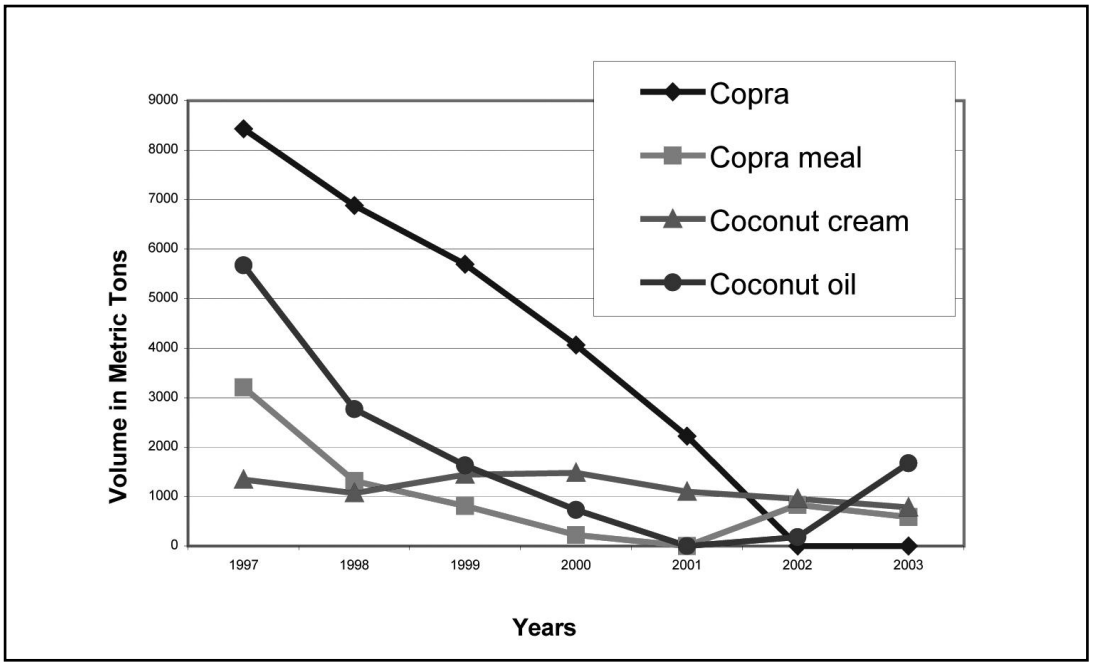

Figure 1.3: Coconut export volume, Samoa 1997-2003
Source: (Cloin 2005b)

1.3 Anaerobic digestion

In addition to being readily available within many PICs, coconut copra is also high in biodegradable organic carbon. One alternative use for this local resource would potentially be the conversion of the coconut copra into a valuable and useful energy source through biological processes, such as anaerobic digestion.

Anaerobic digestion is the process by which a mixed biological culture attacks a complex organic material in the absence of oxygen to produce biogas (Gray 2004). Traditionally the anaerobic treatment process has been used for excess sludge digestion in wastewater treatment plants and for the treatment of strong industrial wastes.

In recent years however anaerobic technology has become more popular due to a) its potential to produce biogas that can be used to generate electricity and b) valuable solids which can be used as a soil conditioner in land applications. With the anaerobic digestion process, a readily available biodegradable organic carbon source is required as an energy source for several groups of carbon-catabolising, anaerobic bacteria species present (Gray 2004).In this context, coconut copra can be regarded as a large potential renewable energy source when applied to biological processes and a potentially local, sustainable and less expensive alternative to fossil fuels. From the perspective of remote Pacific Islands and the people that inhabit them, the application of anaerobic biotechnology could allow increased access to energy for many communities.

The anaerobic digestion process is characterized by a series of biochemical transformations caused by the degradation of organic matter or substrate. The complete process involves several distinct stages; hydrolysis, the acid forming stages of acidogenesis and acetcogenesis and finally methanogenesis (Figure 1.4). The degradation of organic matter to produce the biogas also relies on the complex interaction of several different groups of bacteria with the two main groups being the acid-producing bacteria (acidogens) and the methane-producing bacteria (methanogens) (Fantozzi and Buratti 2009).

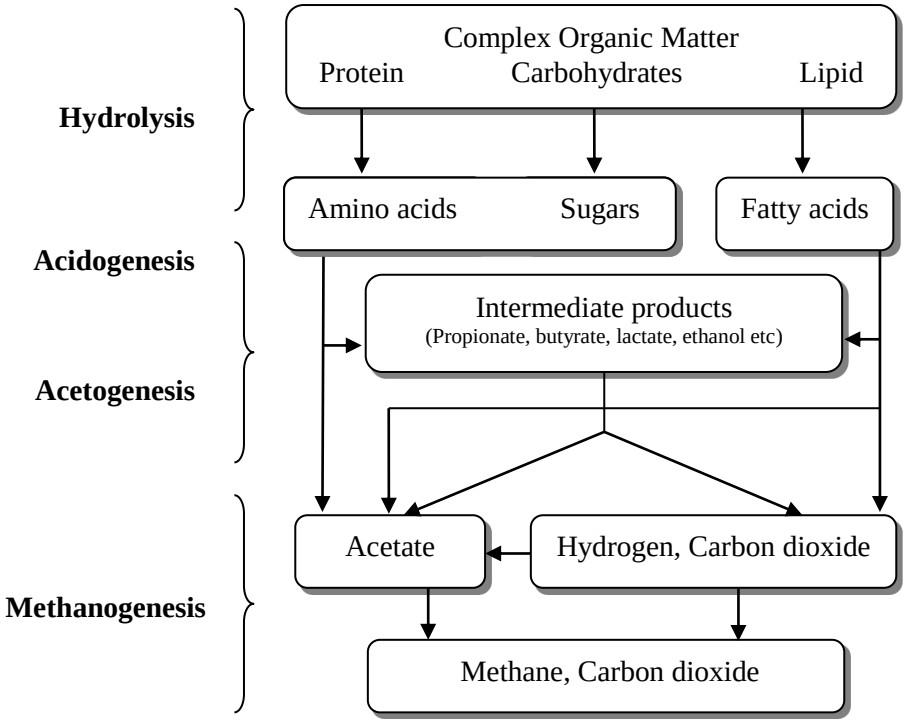

Figure 1.4: Pathways of the anaerobic digestion of organic substrate
Source: Adapted from (Khanal 2008)

In the hydrolysis stage, complex organic compounds such as proteins, carbohydrates and lipids are degraded into soluble products such as simple sugars, amino acids and LCFAs by enzymes excreted by fermentative bacteria (Gray 2004).

The products from this hydrolysis stage are used in the acidogenesis and acetogenesis stages to generate Volatile Fatty Acids (VFAs) which together with ethanol are converted to acetate, carbon dioxide (CO_2), and hydrogen (H_2). These end products are the primary substrates for the methanogenic bacteria to produce biogas which generally consists of 50-75% methane (CH_4), 50-25% CO_2 and trace amounts of nitrogen, hydrogen and hydrogen sulfide.

Approximately 70% of the digester methane originates from acetic acid (CH_3COOH) (equation 1) while the remainder of the digester methane originates from the reduction of CO_2 and hydrogen (H_2) (equation 2) (Chynoweth 1987).

$$Acetic\ acid: \quad C\ H_3\ COOH \ \rightarrow \ CH_4 \ + CO_2 \qquad (1)$$

$$Hydrogen: \quad 4\ H_2 + \ CO_2 \ \rightarrow CH_4 \ + 2\ H_2O \qquad (2)$$

Maintaining the symbiotic relationship between acidogens and methanogens is critical in sustaining the successful operation of any anaerobic digester and failure to achieve this objective is the primary cause of reactor instability (Chen et al. 2008). Any significant changes in environmental conditions can adversely affect the equilibrium between these two groups of micro-organisms, resulting in the build up of VFA intermediates which can inhibit the overall process and result in reduced methane production.

1.3.1 Substrate characteristics and Organic Loading Rate (OLR)

Biogas can be produced from the anaerobic digestion of a number of different substrates. These include domestic sludge (Halalsheh et al. 2005), the organic fraction of municipal solid waste (Zhang et al. 2007) and fruit and vegetable wastes (FVWs) (Bouallagui et al. 2005; Cecchi et al. 1993; Kim et al. 2006; Mata-Alvarez et al. 1992a; Viswanath et al. 1992). In addition, organic wastes from agricultural sectors such as olive pomace (Tekin and DalgIç 2000) and the processing industry have been anaerobically digested (Kryvoruchko et al. 2009).

The amount of substrate feed in into the system is termed the Organic Loading Rate (OLR) and is commonly expressed in terms of kg COD (chemical oxygen demand)/m^3·day, Volatile Solids (VS) / L·day or VS / m^3·day. In single stage, anaerobic digestion of solid wastes, problems may occur if the substrate is easily degraded or the OLR is increased above the systems capabilities. This is because acidogenic activity and the VFA intermediates produced in the acid forming stages, increase at higher OLRs and the slower growing methanogenic population cannot increase their biological activity to the same extent. Substrates that are considered more biodegradable can also result in larger and faster VFA production which can cause a significant drop in pH and gas production (Bouallagui et al. 2005).

The methane yield is measured by the amount of gas that can be produced per unit volume of VS contained in the feedstock after subjecting it to anaerobic digestion for a sufficient amount of time under a given temperature (Zhang et al. 2007). The methane yield is also an indication of the biodegradability of the substrate as feedstock with low VS to Total Solids ratios (VS/TS), such a lignin, are not easily degraded using anaerobic processes therefore the amount of gas produced is also very much substrate dependent.

Dinsdale et al. (2000) examined the methane production of inclined tubular reactors with influent of activated sludge, fruit and a vegetable waste mixture while Bouallagui et al. (2005) investigated digestion of a similar FVW substrate with Continuously Stirred Tank Reactors (CSTRs). The total methane yields achieved were 0.37 m^3/kg ·VS and 0.47 L CH_4/g VS for the tubular and CSTRs, respectively (Bouallagui et al. 2005; Dinsdale et al. 2000).

Zhang et al. (2007) investigated the anaerobic digestion of FVW consisting of two different VS percentages of 74 and 87% using batch reactors operating at a thermophilic temperature of 50 (± 2°C). The average methane yields after 28 days of digestion were 0.425 and 0.445 L CH_4/g VS added, respectively (Zhang et al. 2007).

Verrier et al. (1987) and Mata-Alvarez et al. (1992) both examined the performance CSTRs for the treatment of the solid and organic fraction of vegetable wastes at mesophilic temperatures. The maximum loading rate achieved by Verrier et al. (1987) was 3.6 VS/L·day and the respective methane yield was 0.37 CH_4/g·VS. However, Mata-Alvarez et al. (1992b) reported a maximum specific gas production of 0.762 m^3/ kg VS fed after one month of operation.

There is little research available in the literature on anaerobic digestion using coconut copra as a substrate; however Naksagul et al. (2006) have examined the production of biogas from the treatment of coconut milk wastewater using an Up flow Anaerobic Sludge Blanket reactor (UASB). Their results showed that the average biogas yield was 195 L per kg of COD removed when the digester was operated within the temperature range of 29 - 32° C, a pH of 6.6-7.3, a hydraulic retention time (HRT) of 16-28 hours and an organic loading rate of 0.88 - 1.55 kg COD/m^3 ·day. The average biogas composition of methane, nitrogen, carbon dioxide and other gases consisted of 75.5%, 14.1%, 8.3% and 2.1% respectively (Naksagul et al. 2006).

2 Research objective

The primary objective of this research is to determine the potential for methane gas production from coconut copra. The research is divided into two separate phases; Phase I and II. Phase I consists of initial investigations using tubular batch reactors while Phase II includes laboratory scale, CSTRs. Different OLRs together with other variables (start-up procedure, nutrient supply and mixing frequency) which affect the anaerobic digestion process are investigated during both phases in order to optimize methane production and increase the overall conversion efficiencies of the coconut copra substrate to methane gas.

3 Phase I: Batch Reactors

The biodegradability of the coconut substrate was first determined through a series of batch digestion tests (Phase I). The objective of Phase I was to determine the optimal OLR that could be achieved before proceeding to a more complex CSTR model. It was also used to determine any potential differences between various forms of coconut copra as well as to establish the best pre-digestion preparation of the substrate for optimum biogas production.

3.1 Materials: Inoculum

The inoculum for the batch reactors and CSTRs was sludge sourced from the anaerobic digesters at the Christchurch Wastewater treatment plant (WWTP) located at Bromley, Christchurch. At the time of collection, the digesters were operating at a mesophilic temperature of 36-38°C with an average residence time of approximately 28 days.

Immediately after collection, the inoculum was rested for approximately one week in a temperature controlled room heated to 35°C ± 3°C to ensure all existing internal carbon reserves were exhausted by the micro-organisms present in the sludge. It was noted during the course of the research that there was a slight change in the settling characteristics of the inoculum which may be attributed to process operational changes within the treatment processes at the Christchurch WWTP. The pH and alkalinity for the inoculum were 7.58 ±0.30 and 4,837 mg/L ±290 mg/L as $CaCO_3$ respectively, while the total solids concentration was approximately 1.9% of which 68 % was volatile. A complete summary of the inoculum characteristics are listed in Table 3.1 and Appendix C.

Table 3.1: Characteristics of digested sludge inoculum

Parameters	Units	Mean ± Standard Deviation
pH		7.58 (± 0.30)
Alkalinity	mg/L (as $CaCO_3$)	4,837 (± 290)
Total Solids (TS)	mg/L	18,680 (± 4,230)
	%	1.9
Volatile Solids (VS)	mg/L	12,672 (± 2,321)
Total Suspended Solids (TSS)	mg/L	13,003 (± 1,764)
	%	1.3
Volatile Suspended Solids (VSS)	mg/L	11,747 (± 573)

3.2 Materials: Coconut Copra substrate

The coconut palm requires high humidity (70-80%) and a temperature between 27-32°C for optimal growth therefore palms are not frequently found growing in the southern regions of New Zealand (Santoso et al. 1996). The availability of whole coconut kernels in Christchurch is therefore limited to produce imported from outside the region and are usually available for purchase with the exocarp (outermost layer) removed.

Both raw and processed coconut were tested for TS and VS content using Standard Methods (A.P.H.A 1998). Measured samples were evaporated in weighed dishes in an oven heated to 105°C for 24 hours. The percent TS and percent Moisture Content (MC) for each sample were then obtained by reweighing sample and dishes, where the decrease in weight represented the percent MC and the increase in weight over that of the empty dish the percent TS (equation 3)

$$\%\, MC = \frac{(A-B)}{A} \cdot 100 \qquad \%\ TS = \frac{(A-B)}{(C-B)} \cdot 100 \qquad (3)$$

Where:

A: sample and dish (post-oven) (g)

B: dish (g)

C: sample and dish (pre-oven) (g)

D: residue and dish (post furnace) (g)

The percent VS was then determined by igniting the dried samples in a furnace heated to 550°C for 4 hours before re-weighing. The percent VS was approximately the amount of organic matter for each coconut sample and was calculated as follows (equation 4).

$$\%\ VS = \frac{(A-D)}{(A-B)} \cdot 100 \qquad (4)$$

The above tests confirmed that raw copra has a MC of approximately 50% (Santoso et al. 1996) and both raw and processed copra consist of 98% VS. A complete summary of the characteristic of both raw and dried coconut copra are included in Appendix A.

The coconut substrate used in the batch reactors included raw copra harvested from whole kernels as well as dried copra in both the fine desiccated and shredded forms (Figure 3.1). Whole kernels were purchased from a local produce store, the copra flesh separated from the coconut water and the copra cut into smaller pieces approximately 10 mm by 3-4 mm in size. Dried coconut is readily available from most supermarket stores and is present in the larger shredded form or more commonly as desiccated coconut.

Before commencing reactor trials, dried samples were soaked in water for 24 hours prior to use in order to determine the best preparation procedure (Figure 3.2). A dried coconut to water ratio of 1:1 was adopted which not only ensured the moisture content was consistent for all the coconut samples but also helped solubilise the dry coconut so that it could be easily fed into the CSTRs during Phase II.

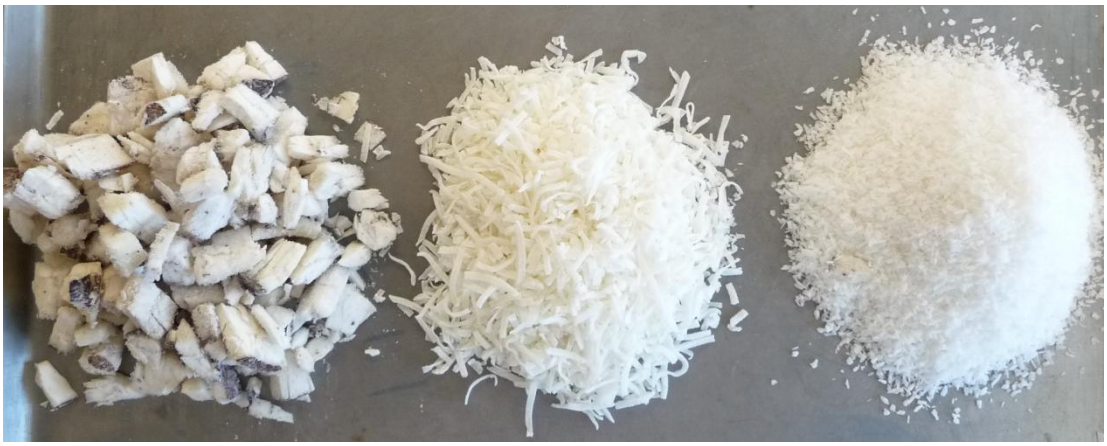

Figure 3.1 Copra substrate 1) wet, raw copra, 2) dried shredded, 3) fine desiccated

Figure 3.2: Different dried coconut, preparation trials;
Water to substrate ratio D) C) 2:1 B) A) 1:1

3.3 Methods: Batch Test Digesters

The batch tests were carried out in digesters made from 3.4 L capacity plastic tubes with air tight seals each filled with 1.5 L of inoculum. The tubes were developed and engineered by the Civil and Natural Resources Laboratory, University of Canterbury, in conjunction with research carried out by Dr Nastein Qamaruz-Zaman. The batch reactors included screw top lids each fitted with both an inlet and outlet valve which allowed the headspace composition to be tested and the biogas to be collected. The digesters were filled with varying quantities and forms of coconut copra (Table 3.2) before the contents were stirred and the lids tightly sealed on each tube. After sealing the lids tightly with a screw-top lid and rubber gasket, anaerobic conditions were established by flushing the headspace of the tubes with nitrogen gas for 20 seconds. A 5 L Tedlar bag (SKC Inc. USA) or gas counter was then attached to the outlet stopper valve of each tube to collect the biogas produced (Figure 3.3 and Figure 3.4).

Table 3.2: Phase I, batch reactors test matrix

Test number	Type of coconut	Abbreviation	Quantity of substrate (g VS)
1	Desiccated fine	DF	102
2	Raw	R	102
3	Desiccated fine	DF	80
4	Desiccated fine	DF	60
5	Desiccated fine	DF	40
6	Desiccated fine	DF	25
7	Desiccated fine	DF	15
8	Desiccated fine	DF	12
9	Desiccated fine	DF	9
10	Desiccated fine	DF	6
11	Raw	R	6
12	Desiccated shredded	DS	6
13	Desiccated fine	DF	3.6
14	Desiccated fine	DF	1.8

Figure 3.3: Elevation view, Batch reactor

Figure 3.4: Plan view, Batch reactor

The incubation period for each set of batch reactors was the same as the HRT and was defined as one test which allowed the anaerobic digestion process to progress continuously from the hydrolysis stage to the methanogenic stage. Each test was operated with a HRT of approximately 8 days, at which point there ceased to be any significant change in the observed gas production.

The batch reactor tubes were stored in a temperature controlled room heated to a mesophilic temperature of 35°C ± 3°C for the duration of each test. The gas composition was determined by measuring the headspace of each tube with a landfill gas analyser (Geotech GA 2000 Landfill Gas Analyser). The percentage of CH_4, CO_2, oxygen (O_2) and other gases (nitrogen and other trace gases) were recorded daily by connecting the gas analyser to both the inlet and outlet for each tube and operating the pump for approximately 50-60 seconds. Each tube was mixed daily before testing the headspace by gently swirling the bottom in a circular motion for approximately 10 seconds. Daily mixing of the batch reactors promoted

contact between the coconut substrate and biomass and also ensured that the gas was released from the liquid for an accurate gas composition reading.

Initially, 5L Tedlar bags (SKC Inc. USA) were attached to an outlet ports near the top of each tube and a manual water replacement method was used to measure the amount of gas produced daily. The Tedlar bags were later replaced by electronic gas counters developed by in house technicians at the University of Canterbury (Figure 3.5). The gas counters operated by measuring the total time taken to displace a known volume of water by the contained gas output from each reactor.

The gas counters worked on the principle that a "U" shaped tube was open to atmosphere on one end and sealed with a solenoid operated valve on the other. The biogas generated inside each reactor was piped into the sealed end of the tube through the valve. As the gas built up, it displaced the column of water which was kept at a constant amount of 100mL for repeatability. The displaced water triggered a connection between two electrodes which was recognised by the embedded logger.

The counter then recorded the amount of time (seconds) it took for the water to be displaced, logged it and then activated the valve on the sealed end of the tube to release the built up gas to atmosphere allowing the tube to come to a state of equilibrium before repeating the process. Each gas counter was calibrated against known volumes of air prior to use and data logged from each counter was used to determine the rate of gas production for each reactor.

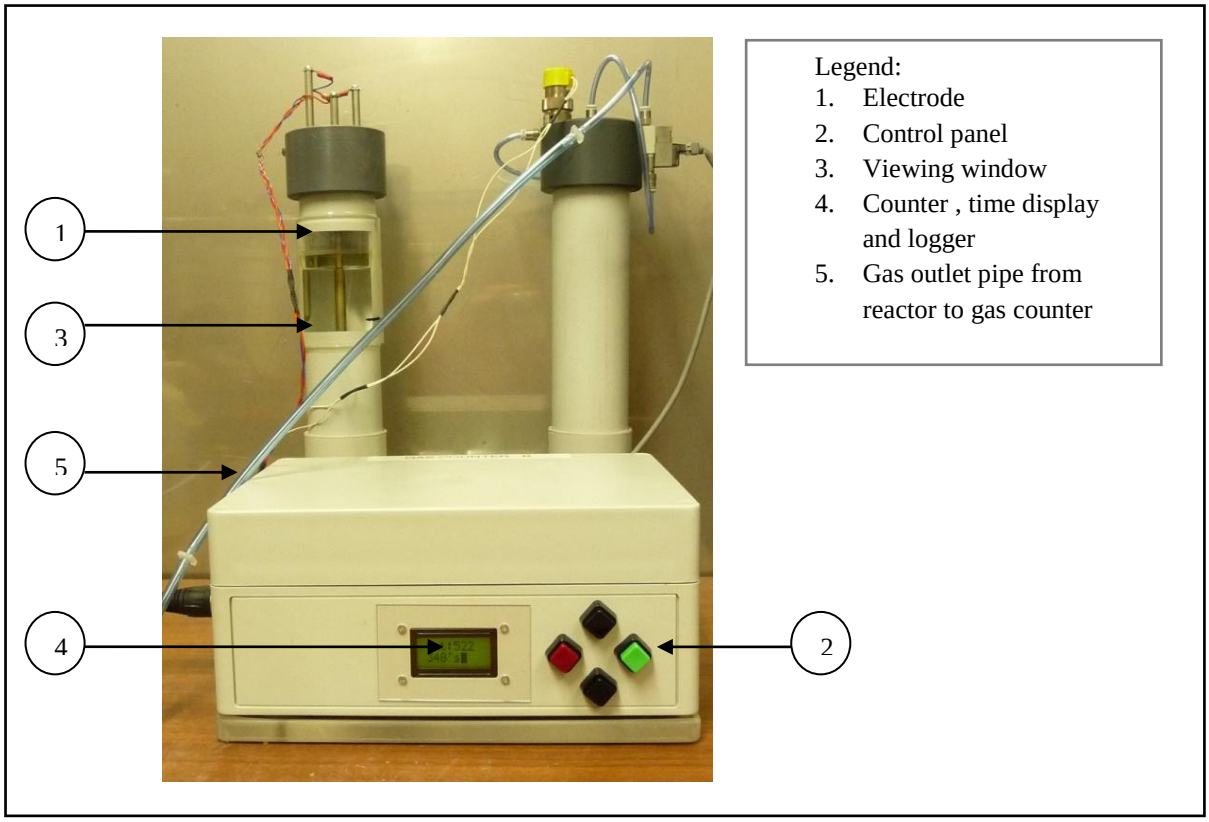

Figure 3.5: Gas counter B

Duplicate tubes were included in each test from which a mean and standard deviation value for each measured parameter could be determined. Control tubes containing inoculum were also included in each test to measure the background methane production from the carbon sources initially present and this volume was subtracted from the total gas production.

At the conclusion of each test, the pH of each reactor was measured using a pH probe (EDT RE357 Microprocessor pH meter). The pH probe was calibrated regularly using known standard solutions at pH 4, 7 and 10 (Biolab, Australia) according to the manufacturer's specifications in order to ensure confidence in the results and indentify deterioration in the probe itself.

The alkalinity levels were also measured by a manual titration method using a H_2SO_4 titrate with a concentration of 0.1 N and methods as specified by the Standard Methods (A.P.H.A 1998) (equation 5).

$$Alkalinity\ \left(CaCO_3 \frac{mg}{L}\right) = \frac{A\ \cdot N\ \cdot 50000}{sample\ amount\ (mL)} \qquad (5)$$

Where:

A: volume of standard of acid used in titration (mL)

N: normality of standard acid (0.1 N)

4 Phase II: Semi - Continuously stirred tank reactors (CSTR)

Phase II of the research progressed the knowledge gained from the batch reactors by investigating the biogas potential of coconut copra using CSTRs. Although CSTRs are considered more complex systems, they have various advantages over batch reactors including increased contact between biomass and substrate as well as greater dispersion of toxic concentrations as a result of either semi-continuous or continuous mixing processes. In addition, continuous flow systems typically produce higher quantities of biogas than batch systems and are generally able to tolerate higher OLR (Khanal 2008).

4.1 Materials: CSTRs

Two identical CSTRs (Reactor 1 and 2) were established for Phase II. The stainless steel reactors were approximately 30 L in volume and featured removable lids which were able to be closed with screw fixtures and sealed with silicone to establish airtight conditions. The inoculum used for Phase II was sourced from the same location as Phase I (Section 3.1). Similar to the batch reactors, each CSTR was filled with inoculum and the lid sealed firmly before anaerobic conditions were established by flushing the headspace of each reactor with nitrogen gas. A wasting port on the side of each reactor was set at a constant overflow position therefore ensuring that the active volume remained at a constant 20 L each time the reactor was fed and wasted (Figure 4.1 and Figure 4.2).

Figure 4.1: Reactor 2 and Gas counter B

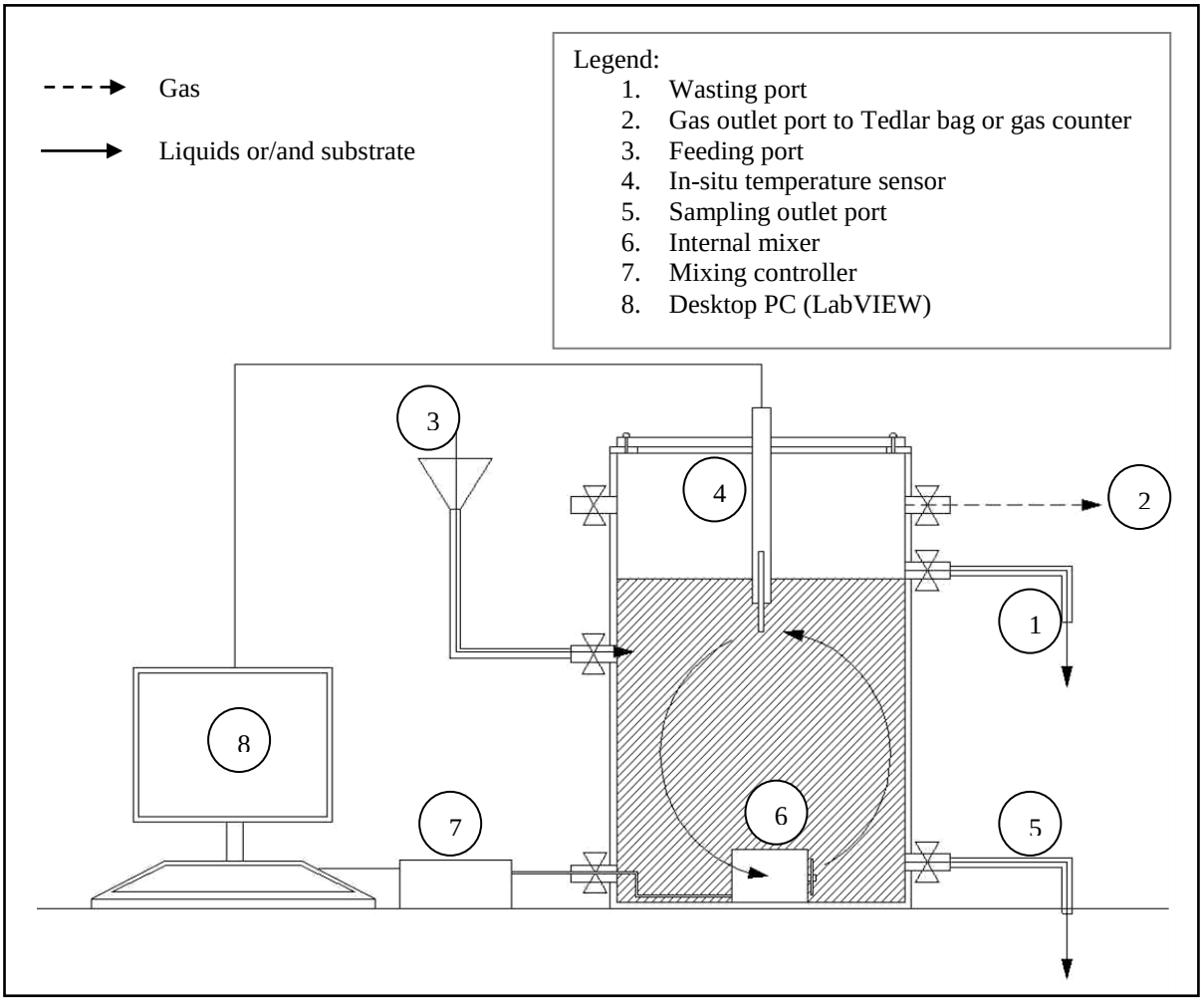

Figure 4.2: Schematic diagram experimental setup for CSTRs

Mixing for each reactor was carried out by propeller stirrers (Model 2E-38N, Little Giant Pump, Oklahoma) which were fixed internally to the base of each tank. Each mixer was initially connected to a digital timer (Compact digital time switch PC697 Series 3, Arlec, Australia) which allowed the stirrers to be switched on and off automatically from the mains power supply at the required time intervals. The electronic timers were later replaced with a LabVIEW computer software package (National Instruments 2007) controlled through a desktop PC which allowed greater flexibility with respect to management of the mixing regime. Continuous mixing of the CSTRs could not be achieved as the heat generated from the internal stirrers significantly increased the internal temperature of the reactors to above 47°C and outside the desired mesophilic temperature range (30 -35 °C). The stirrers were therefore operated semi-continuously to prevent any temperature fluctuations as it is recommended that anaerobic systems should be designed for temperature variations not exceeding 0.6-1.2 °C (Khanal 2008).

Each run operated between 17-104 days and was defined as the period between the day of initial substrate feeding to the day which the reactors had stopped producing regular amounts of biogas, at which time the feeding procedure was discontinued. Similar to the batch reactors, each run allowed the anaerobic digestion process to progress continuously from the hydrolysis stage to the methanogenic stage. During each run, an environmental or operational parameter was investigated in order to maximise methane production and determine the optimal OLR using the CSTR systems (Table 4.1)

The CSTRs remained in a temperature control room heated to 35°C ± 3°C for the duration of reach run and the internal temperature was logged continuously with an in-situ, solid-state sensor in order to monitor any temperature fluctuations.

Table 4.1: Phase II Run and parameter matrix

Run number	Summary of parameters investigated
1	Preliminary run testing HRT/SRT
2	Secondary run and adjustment of HRT/SRT
3	Effects of accelerated start-up
4	Effects of additional nutrient supplements
5	Effects of mixing frequency

4.2 Methods: CSTRs

4.2.1 Sampling and feeding procedures

Similar to Phase I, the amount of gas produced from each CSTR was recorded daily either through Tedlar bags and a manual water displacement method or data logged through the electronic gas counters. The head space of each reactor was also read daily using a landfill gas analyser (Geotech GA 2000 Landfill Gas Analyser) and the percentage of CH_4, CO_2, and other gases recorded. This allowed the amount of methane and rate of methane production to be calculated at each OLR increment.

The pre-digestion substrate preparation for the CSTRs also remained identical to the procedure established for the batch reactors where approximately 24 hours prior to feeding, measured quantities of desiccated coconut were soaked in water in order to reconstitute the dried coconut to the equivalent moisture content of raw coconut (Section 3.2).

Prior to commencing feeding, the outlet gas collection port on each CSTR was closed and a portion of liquid was wasted of which 100 mL was set aside for sampling. The remaining effluent was then mixed with the prepared substrate immediately before feeding and an additional quantity of water was added to achieve the required HRT. Both the inlet feeding

port and outlet wasting ports were opened and the coconut mixture was then poured into each reactor through a funnel before both ports were then closed tightly again. The feeding port and wasting ports were located at different sides of the reactors in order to prevent short circuiting of the substrate during the feeding procedure (Figure 4.2).

Alkalinity, pH and TS tests were completed daily on the sludge samples taken from both reactors after the feeding and wasting procedures were completed. Samples from selected runs were also tested for Ammonia (NH_3-N) concentration levels using the Hatch 'N Tube method (High Range 0-50 mgL). Sludge samples were centrifuged for 8.5min at 4400 rpm (Eppendorf, Centrifuge 5702), filtered through a glass micro fibre filter (GF/C) and diluted with deionised water before being combined with both Ammonia Salicylate and Ammonia Cyanurate reagents.

After a 20 min reaction time, the samples were then measured using a spectrophotometric method (Hach Spectrophotometer, wavelength 655nm, Method # 343). In addition, samples were tested for Soluble Chemical Oxygen Demand (SCOD) at each OLR using the Hatch Dichromate Reactor Digestion Method test (Section 5.2).

4.2.2 Start up procedure

The start-up of a reactor is the initial commissioning period during which the reactor is brought to a point (steady state) where the normal performance of the biological system can be achieved with continuous substrate feeding (Khanal 2008). Achieving this steady state condition is important for anaerobic micro-organisms, especially methanogenic bacteria, which have slow growth rates in comparison to aerobic microorganisms and are particularly susceptible to changes in environmental conditions. Poor start-up in biological treatment systems can also lead to prolonged periods of acclimation and ineffective removal of organic matter (Angelidaki et al. 2006).

It is recommended by the Water Pollution Control Federation (WPCF) that the feed load during the start up period should be approximately 20 % of the anticipated daily loading capacity (WPCF 1987). A start-up loading was therefore obtained by applying 20 % of the average optimal OLR determined from the batch reactors to the 20 L active volume of the CSTRs. The reactors were maintained at this OLR until steady state was observed (ie: defined as less than 10 % variation in both gas production and methane percentage in addition to stabilised pH and alkalinity). In Run 3, an accelerated start-up procedure was also investigated where the OLR was increased rapidly to determine if the time period for the system to reach steady state could be significantly reduced.

4.3 Operating conditions

4.3.1 Hydraulic Retention Time (HRT) and Organic Loading Rate (ORL)

The HRT indicates the time the waste remains in the reactor in contact with the biomass (Khanal 2008). In flow-through systems without recycle, such as the CSTRs adopted in Phase II, the HRT and retention time of the microbial biomass or sludge (SRT) are the same. In situations when the influent streams contain high solids concentrations longer retention times are required in order to maximise bioenegry production (Khanal 2008). An initial HRT of 24 days was adopted for Run 1 based on continuous systems with similar FVWs (Mata-Alvarez et al. 1992b; Viswanath et al. 1992). However, with the primary research objective in this research being to maximise methane production, longer HRTs (up to 200 days) are possible since a field operator would not want to be concerned with sludge wastage - just gas production.

OLR is the amount of substrate fed into the system and is generally expressed in terms of kg per Chemical Oxygen Demand (COD) or VS/L·day or VS/m^3·day. In a stable system, the hydrogen and the VFAs formed by the acidogenic bacteria are consumed at the same rate by the methanogens. When the OLR is increased, the acidogenic activity, which includes the production of mainly VFA, CO_2 and H_2, also increases which can result in an accumulation of organic acids and sudden decrease in pH. Growth of the methane producing methanogens is inhibited below a pH of 6.6 (Gray 2004). Determining the correct OLR for a particular substrate is therefore critical to the optimization of reactor performance and maximising methane production.

In this research, the OLR was systemically increased by increments of 12 g of VS and operated to steady state (usually a period of approximately 12.5% of the HRT). Reactor 1 (Rctr 1) was also operated behind Reactor 2 (Rctr 2) with respect to time of the OLR

increases so in the event of failure of Rctr 1 from substrate overloading the other could still continue at a reduced rate, instead of a complete restart for both systems. The methane production rate, pH, alkalinity, SCOD, VFA production and TS were also closely monitored at each OLR in order to determine how the increase in OLR affected each parameter.

4.3.2 Nutrient supplements

As with all biological systems, anaerobic digestion requires both macronutrients (nitrogen and phosphorus) and micronutrients (trace metals) in addition to a carbon source to promote biomass growth and to sustain the biological process. The required amount of each macronutrient is usually specified as a theoretical minimum carbon to nitrogen to phosphorus (C:N:P) ratio. Some research recommends a C:N:P of 100-128:4:1 (Bouallagui et al. 2003) while other research suggests a C:N ratio between 20-30:1 for optimal biogas generation (Viswanath et al. 1992).

In order to determine the initial C:N ratio of the substrate, samples of dried coconut were sent to an external, accredited laboratory for total carbon (TCOD), total nitrogen (TP) and total phosphorus (TP) analysis (Table 4.2). Since prior analysis had determined that coconut copra was extremely volatile (98% VS) it was assumed that all the carbon within the substrate was available for biological uptake during the anaerobic digestion process. To determine the optimal C: N: P ratio the TCOD content of desiccated coconut was therefore used in preference to SCOD. From the results of the external analysis a C:N:P ratio of 194:6.25:1 for dried coconut was calculated. This ratio was reasonably close to both the recommended C:N:P of 100-128:4:1 for vegetable biomasses to methane (Bouallagui et al.

2003) and also the C:N ratio of between 20-30:1 (Viswanath et al. 1992) with the rate limiting nutrient being phosphorus for this particular substrate.

Table 4.2: Macro nutrient composition dried coconut

Parameter	Units	Value ± detection limits attainable
Total Chemical Oxygen Demand (TCOD)	g O_2/100g	37 (± 2)
Total Nitrogen (TN)	g/100g	1.24 (± 0.02)
Total Phosphorus (TP)	g/100g	0.190 (± 0.001)

Substrates are often supplemented with additional nitrogen, in the form of urea or ammonium chloride, and phosphorus, as phosphoric acid or a phosphate salt, to promote biomass growth and stabilize digester process efficiency (Lane 1984). Thus, in this research investigations also included the supplementation of the coconut substrate with diammonium hydrogen phosphate, $(NH_4)_2HPO_4$,which was added during Run 4 in order to determine if biological activity and methane production could be further maximised.

4.3.3 Alkalinity

Maintenance of bicarbonate alkalinity greater than 2,500 mg/L as $CaCO_3$ should assist with digester stability (Chynoweth 1987). This is especially important for substrates high in lipids, such as coconut copra, since the lipid fraction is hydrolysed to LCFAs and VFAs which if allowed to accumulate becomes toxic to methanogens (Dinsdale et al. 1996). Sodium bicarbonate or lime if often added to both lab and full scale digesters in order to act as a buffering agent and better control the concentrations of organic acids. The addition of alkalinity in the form of bicarbonate was only investigated during Run 3 and 4 where rapid decreases in pH levels were experienced.

5 Volatile Fatty Acids (VFA) and Soluble Carbon Oxygen Demand (SCOD) Track Studies

5.1 Volatile Fatty Acids (VFAs)

VFAs are the intermediates formed during the anaerobic fermentation of organic materials which are converted to methane through biological processes (Section 1.3). The presence of excess VFA concentrations within a reactor is therefore an important indication if the relationship between the acid forming bacteria and acid consuming bacteria (ie methanogens) is balanced.

For a healthy operating anaerobic system, the effluent VFA concentration ranges are generally < 250 mg/L as acetic acid (Khanal 2008). If the symbiotic relationship between the acidogens and methanogens becomes unbalanced due to toxicity from heavy metals, deficiency of nutrients or overloading of substrate, there is an excess accumulation of VFAs which subsequently causes a corresponding decrease in pH and eventually reactor failure. When the total concentration of VFAs exceeds 3,000 mg/L or the propioinc acid concentration becomes higher than 300 mg/L, an inhibition of the anaerobic digestion process can take place (Kryvoruchko et al. 2009).

The most dominant VFAs usually present during the anaerobic digestion process are acetic ($C_2H_4O_2$), propioinc ($C_3H_6O_2$) and butyric ($C_4H_8O_2$) which are reported as mg/L. Determination of these individual VFAs concentrations is also useful, as a shift from lower to higher carbon acids, such as acetic to butryic, may be an indication of reactor instability (Gray 2004).

In order to determine the concentration of the VFAs in the batch reactors during Phase I, digested sludge samples were analysed at the conclusion of each test.

Similarly for the CSTRs, digested sludge samples of approximately 50 mL were taken at 30 min time intervals before (and for 3 hours immediately after) feeding at each OLR. The sludge samples from both the batch reactors and CSTRs were firstly centrifuged for 8.5 min at 4400 rpm (Eppendorf, Centrifuge 5702), before filtered through a glass micro fibre filter (GF/C) followed by a 0.45 μm syringe filter. The filtered supernatant samples were then analyzed by a gas chromatograph (HP 6980) of which conditions and methods are listed below in Table 5.1.

Table 5.1: Gas chromatograph method analysis

Column	HP 19091 N-133, HP INNOWax Polyethlene Glycol (30m x250μm x 0.25 μm)
Gas	Nitrogen. Flow rate: 2.2 mL/min Pressure: 27.06 psi
Oven	120°C for 1 min then 10°C/min to 250°C hold at 250°C for 2 min, temperature decrease to 120°C hold for 0.5 min
Detector	FID at 300°C
Analysis time	18 min

The concentration of each VFA corresponds to the area underneath peaks which register at particular retention times along the gas chromatograph (GC) response curve. The species and concentration of each VFA present in the filtered samples were able to be identified by comparing the areas recorded underneath each peak to calibration curves established for standard samples of acetic, propioinc, butyric and also isovaleric acids (Appendix H).

In order to ensure the reliability of the gas chromatograph results, spikes containing known concentrations of acetic acid were included within the filtered sampling series. The GC results for the spikes showed a corresponding retention time as the standards to give confidence to the tested samples (Figure 5.1 and Figure 5.2).

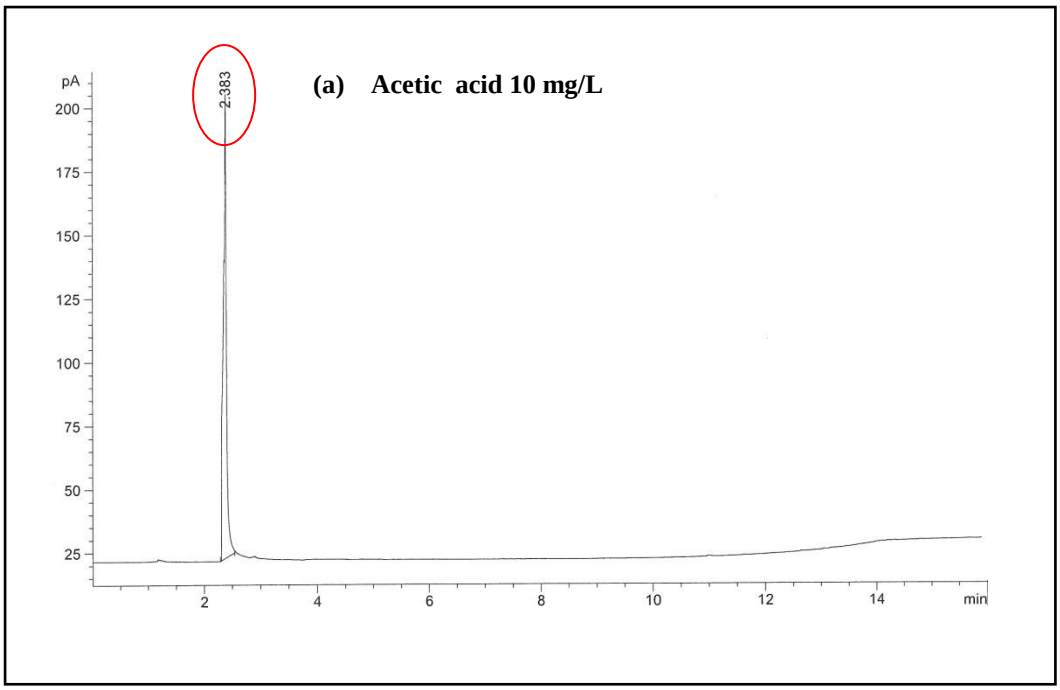

Figure 5.1: Spike sample GC results

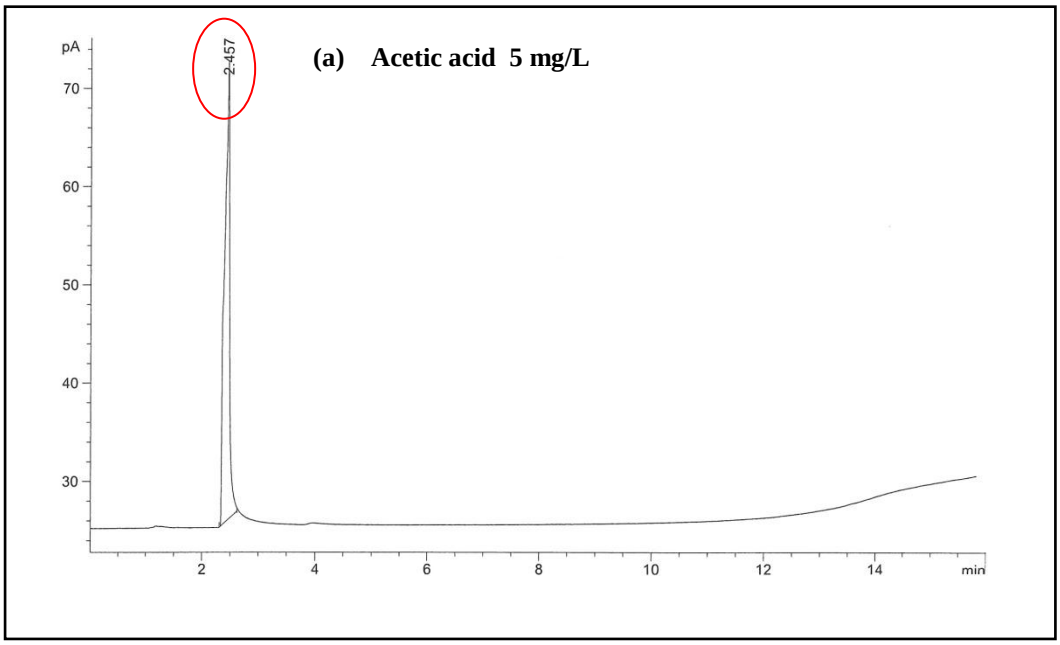

Figure 5.2: Calibration sample GC results

5.2 Soluble Chemical Oxygen Demand (SCOD)

The concentration of SCOD is also an important indication of a healthy reactor system as the presence of excess SCOD in the effluent suggests that the organic substrate is not being effectively converted into methane and carbon dioxide. SCOD levels for an unhealthy reactor system are usually extremely high however exact threshold levels are also dependant on pH, temperature and also the presence of other inhibitors such as concentrations of ammonia and toxic metals.

The filtered sludge samples from both the batch reactors and CSTRs were analysed for SCOD using the Hatch Dichromate Reactor Digestion Method test. This method required the filtrate for each sample to be diluted with deionised water to an appropriated range (0-1,500 mg/L) before small quantities were added to vials containing measured amounts of the reagent (potassium dichromate, $K_2CR_2O_7$). The vials were then digested at 150° for 120 minutes before they were measured using spectrophotometric methods (Hatch Spectrophotometer, colorimetric wavelength 650nm, Method # 435). Included in each sample series was a quality control standard of known concentration to ensure confidence in the sample results.

6 Results and Analysis

6.1 Phase I: OLR and Methane Production

6.1.1 Determination of optimal OLR

The initial OLR for Phase I was estimated based on the optimal rate achieved with a mixed FVW using similar tubular reactors (Qamaruz-Zaman 2010). Tests 1 and 2 included both desiccated fine (DF) and raw (R) quantities of coconut copra substrate at an OLR of 102g VS (20.13 kg COD/m^3·day). A total of 8.17 L and 8.04 L of gas were produced from the desiccated and raw samples respectively however, the majority of the gas produced was CO_2 and only an average of 12.5 % and 14.3 % of the gas proportions were the desired CH_4 (Table 6.1 and Figure 6.1). At the conclusion of tests the pH had decreased considerably from an influent pH 7.80 to an effluent pH 5.33 and 5.50 for both the desiccated and raw substrate, respectively (Table 6.2).These acidic pH levels were outside the recommended optimal pH range of 6.6-7.4 for the growth of methanogenic bacteria which coupled with the strong odour omitted from the effluent, indicated souring from substrate overloading had occurred (de Lemos Chernicharo 2007). The OLR was therefore systematically decreased in the following tests and limited to only the desiccated coconut type, due to both the availability of whole coconuts and also time required for substrate preparation. The raw copra would then be reintroduced and tested at the OLR at which methane production was optimised using the desiccated copra substrate. Tests 3, 4 and 5 (OLRs of 80, 60 and 40 g VS) had negligible methane yields, low methane percentages and effluent pH readings of pH 5.35, 6.05 and 6.20 respectively, suggesting these OLRs were also too high to successfully sustain a stable methanogenic population (Figure 6.1, Figure 6.2 and Table 6.1). Tests 6 and 7, consisting of OLRs of 25 and 15 g VS, were also unsuccessful in producing significant methane yields and the average CH_4 percentages remained low at 10.3 and 11.8 %, respectively (Table 6.1 and Figure 6.2).

Table 6.1: Bach reactors methane percentage and yield Tests 1-14

Test number	OLR (g/VS)	Total biogas yield (L)	Methane percentage (%)		Methane volume (L CH_4)		Methane yield (L CH_4 / g VS)	
			Mean	±Stdev (2)	Mean	±Stdev	Mean	±Stdev
Control (no copra)	-	-	8.2	3.1	-	-	-	-
1	102	8.17	12.5	4.1	1.024	0.332	0.010	0.003
2	102	8.04	14.3	6.5	1.150	0.522	0.011	0.005
3	80	6.90	14.2	5.6	0.979	0.383	0.012	0.005
4	60	6.20	15.1	7.6	0.934	0.470	0.012	0.008
5	40	2.60	11.4	5.9	0.297	0.154	0.007	0.002
6	25	1.70	10.3	3.1	0.175	0.053	0.004	0.001
7	15	1.85	11.8	3.3	0.218	0.060	0.013	0.007
8	12	1.80	10.5	3.0	0.188	0.055	0.016	0.005
9	9	1.50	17.0	3.2	0.223	0.048	0.025	0.005
10	6	3.50	47.6	23.6	1.665	0.827	0.277	0.138
11	6	2.55	44.2	20.0	1.128	0.511	0.188	0.085
12	6	1.10	11.4	3.4	0.125	0.037	0.021	0.006
13	3.6	3.70	40.8	17.0	1.511	0.628	0.420	0.174
14	1.8	1.10	13.8	6.6	0.152	0.073	0.084	0.003

Table 6.2: pH and Alkalinity at conclusion of Tests 1-14

Test number	OLR (g/VS)	pH		Alkalinity (mg/L $CaCO_3$)	
		Mean	±Stdev	Mean	± Stdev
Control	-	7.70	0.15	4662	196
1	102	5.33	0.14	1510	42
2	102	5.50	0.14	1565	64
3	80	5.35	0.07	2395	92
4	60	6.05	0.07	2770	14
5	40	6.20	0.14	2590	57
6	25	6.40	0.14	2587	47
7	15	6.95	0.03	3150	64
8	12	6.95	0.07	3840	127
9	9	6.52	0.46	4285	233
10	6	7.12	0.18	4270	127
11	6	7.24	0.05	2238	513
12	6	7.00	0.14	1988	159
13	3.6	7.31	0.08	5235	233
14	1.8	7.65	0.13	4505	219

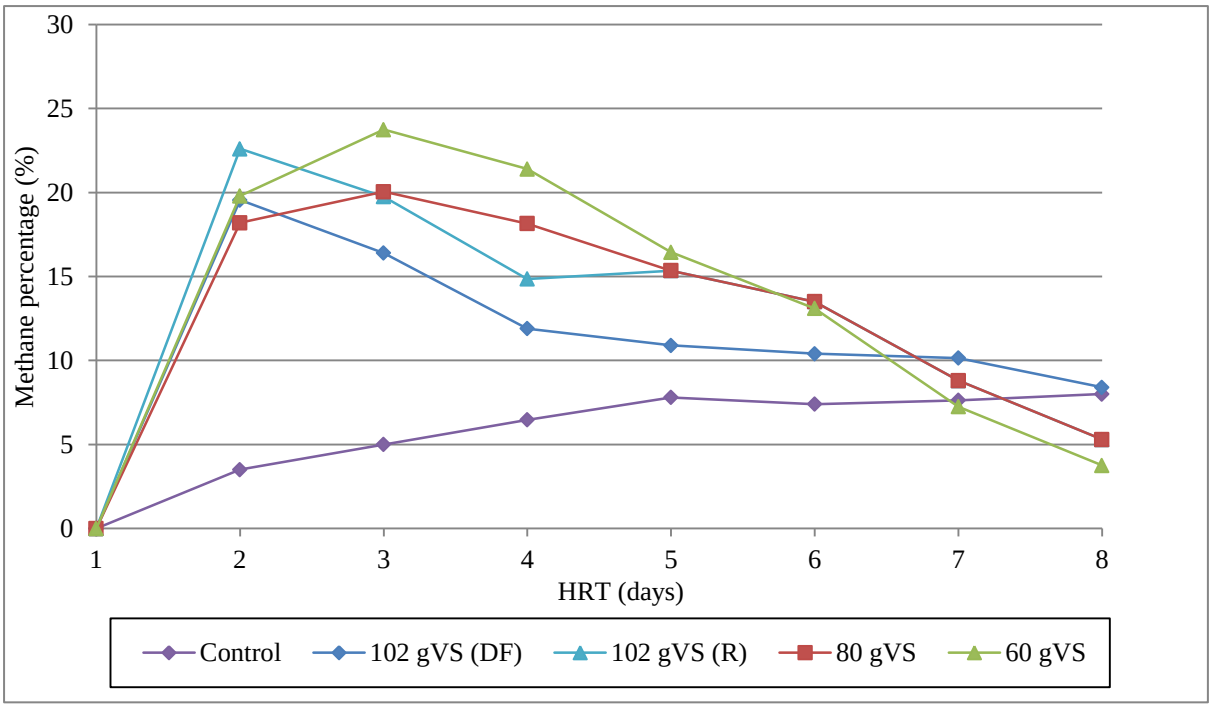

Figure 6.1: Control and Test 1, 2, 3 and 4 methane percentage

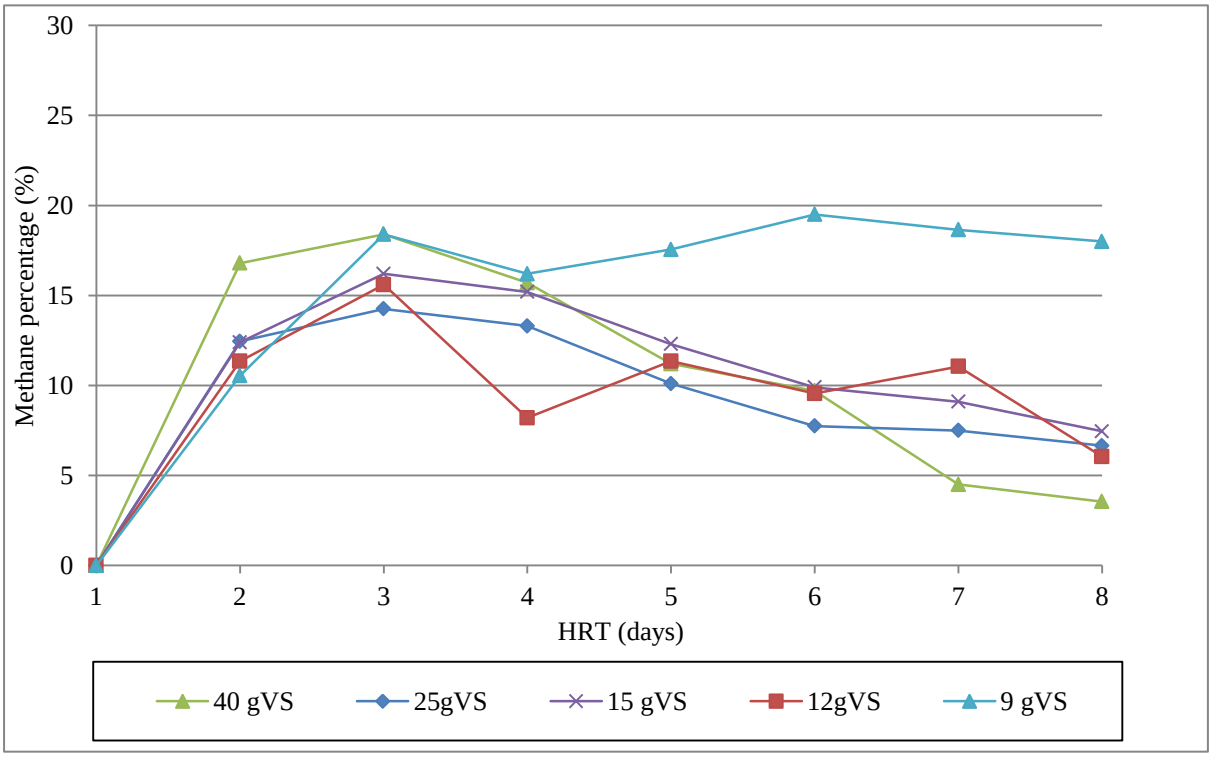

Figure 6.2: Tests 5, 6, 7, 8 and 9 methane percentage

At the OLRs of 12 and 9 g VS (Tests 8 and 9) there was some gas yield in comparison to previous tests. Although the overall average methane percentage remained low for both loading rates at 10.5 and 27.6 % respectively, there was an observed trend of a stabilising methane composition at the OLR of 9 g VS where previous runs had displayed decreasing trends after a HRT of 3 days (Figure 6.2). Although the concluding pH at the OLR of 9 g VS was slightly acidic at 6.52 the alkalinity remained high at 4,285 mg / L as $CaCO_3$ (Table 6.2). This high concentration of alkalinity insured sufficient buffering capacity to stabilize the system within the extreme limits of anaerobic treatment demonstrated by the minimal methane yield of 0.103 L CH_4/g VS (Table 6.1).

The OLR of 6 g VS (Test 10) demonstrated a significant increase in both methane percentage and yield over previous tests and achieved an average of 47.6 % and 0.277 L CH_4/g VS, respectively (Table 6.1). Two different types of coconut copra substrate, R and dried shredded (DS), were therefore reintroduced and also tested at this OLR of 6 g VS during Test 11 and 12. The R sample did not reach the same methane percentage average as the DF coconut however the mean methane percentage for the R coconut of 44.2 % was close to the percentage achieved with the DF coconut suggesting a good correlation between the two types. In addition, there was an observed increasing trend of methane production during day 6-8 of the incubation period (Figure 6.3).This may be attributed to the extended hydrolysis period required to degrade the larger particle size of the raw coconut in comparison the finer, desiccated substrate. The shredded coconut produced negligible quantities of gas and the average methane percentage remained low at 11.4% in comparison to other copra types (Table 6.1).

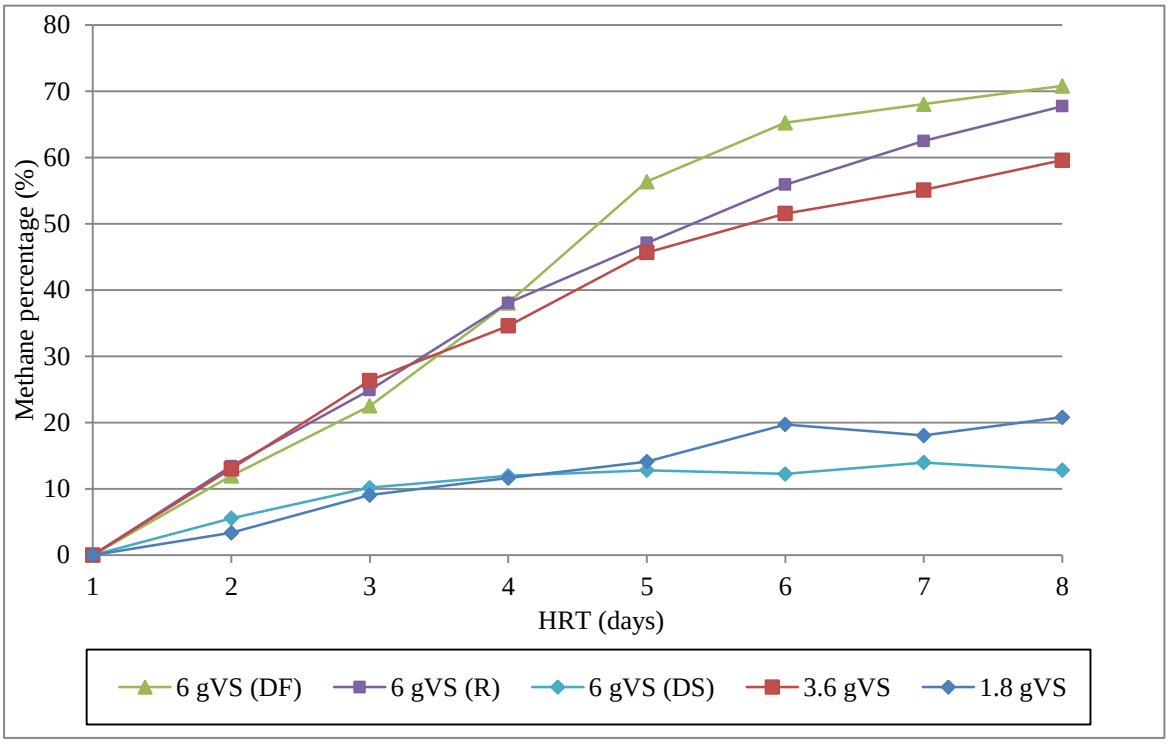

Figure 6.3: Tests 10, 11, 12, 13 and 14 methane percentage

The maximum average methane production of 0.420 L CH_4/g VS was achieved during Test 13 at an OLR of 3.6 g VS (2.4 g VS/L Rctr). At this OLR there was a minimal decrease in the effluent pH to 7.31 and a recovery of alkalinity of 398 mg/L as $CaCO_3$ from the initial inoculum value was also observed (Table 6.2). At an OLR of 1.8 g VS (Test 14) the quantity of substrate available appeared to be the limiting factor in the anaerobic process as the methane composition remained only at an average of 13.8 % and overall methane yield remained minimal at 0.084 L CH_4/g VS. The high alkalinity and pH also remained similar to the control sample at pH 7.65 and 4505 mg / L as $CaCO_3$, respectively (Table 6.1 and 6.2).

The methane production rate per reactor volume, expressed as L CH_4/ L Rctr·day for Tests 8-14 using DF coconut substrate has been summarised in Figure 6.4 below.

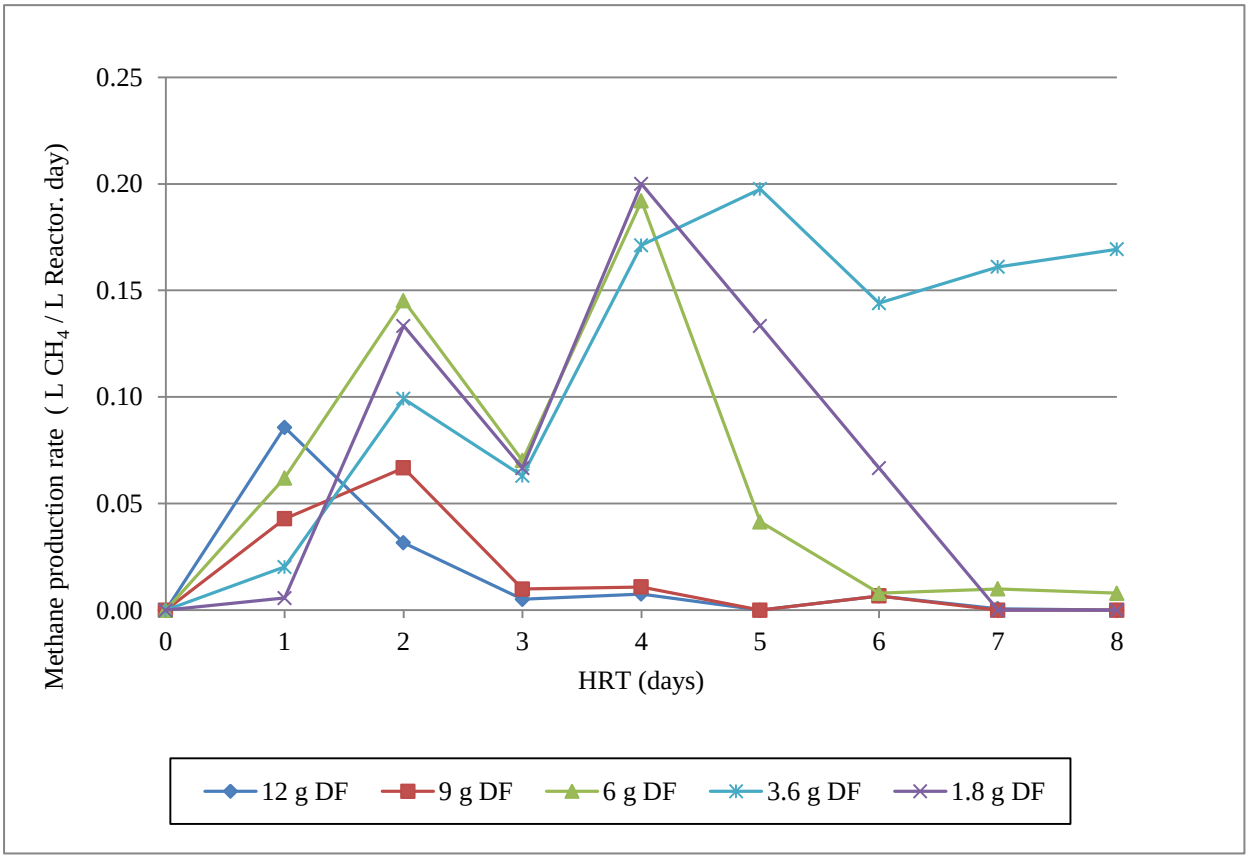

Figure 6.4: Tests 8-14 (DF) methane production rate

The results demonstrate that the maximum methane production rate was 0.200 L CH_4/L Rtr·day which was achieved at the OLR of 1.8 g VS. It was observed that the majority of the gas production and higher methane percentages for both Tests 1-8 were achieved during the first 3 days of the incubation period after which the methane percentages decreased rapidly. At the lower OLRs in Test 8-14 the observed trend of methane percentage was either a fluctuating or gradual increase as the incubation period progressed with a maximum of 71.8 % achieved at an OLR of 6 g VS on day 8 (Test 10) (Figure 6.3). The maximum average methane percentage of 47.6 % (Table 6.1) obtained is comparable to digestion of similar FWVs. Kryvoruchko et al. (2009) achieved an average methane percentage of 50.8 %

with a horizontal plug flow system and potato based substrates digested for incubation periods of 28-38 days while Bouallagui et al. (2005) reported a higher value of 58% with a HRT of 12 days using semi-continuous stirred tubular reactors and a mixed FWV.

All tests, excluding the control, demonstrated a decrease of methane production at a HRT of 3 days however only Tests 10, 13 and 14 recovered from this decrease on day 4 (Figure 6.4). This lag period in methane yield may represent an increase of acidogenic activity resulting in a corresponding concentration increase of organic acid intermediates. At OLRs exceeding a system's capabilities, the intermediates acids produced are unable to be utilized by the methanogenic population at the same rate of production which could contribute to the failure of reactors early into the incubation period at the higher OLRs. The total methane yield for each OLR was also normalised by the quantity of VS added in order to compare each run directly (Figure 6.5). The highest methane production per g VS observed was 0.420 L CH_4/g VS (0.372 CH_4 m^3 at STP/ kg VS) which was achieved at the OLR of 3.6 g VS (Test 14) while at the OLR 6g VS (Test 10) the average methane production was 25% lower at 0.277 L CH_4/g VS .

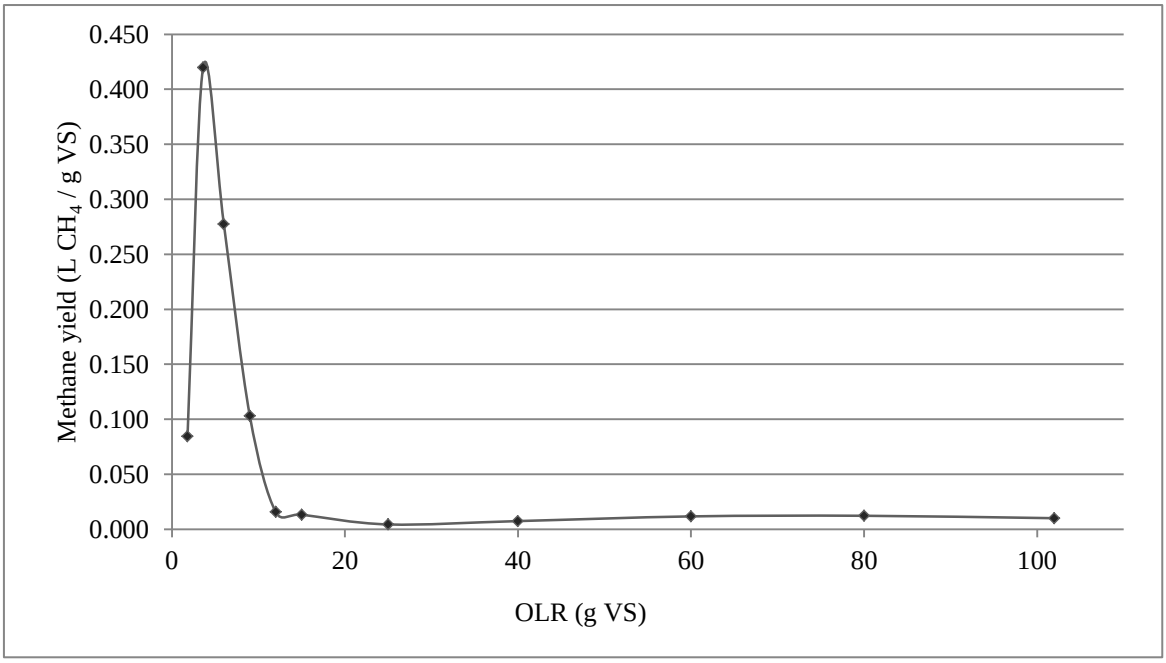

Figure 6.5: Methane yield per gram VS Test 1-14 (DF)

The maximum methane yields achieved at the OLRs of 3.6 and 6g VS are near the specific yields of 0.332 and 0.377 L CH_4/g VS reported by Krvoruchko et al (2009) with potato pulp and peel substrates. The VS content of the coconut copra substrate (98 % VS) is also similar to that of spent beet pulp (96% VS) digested in single batch, leech-bed reactors as part of investigations by Koppar and Pullammanappallil (2008). Their average methane yield was 0.336 m^3 CH_4 at STP/kg VS which is also comparable to the maximum yield achieved with the copra substrate. In addition, it was noted that 95% of the theoretical methane potential using the spent beet substrate was achieved within the first 8 days of the incubation period. The maximum methane yield achieved with the batch reactors was slightly lower than the values reported by Qamaruz-Zaman (2010) who obtained a maximum of yield of 0.429 L CH_4/g VS at an OLR of 18.8 g VS/L using similar tubular batch reactors. It should be mentioned however this value was achieved after an incubation period of 60 days while at 6 days, the methane yield was reported as 0.326 L CH_4/g VS.

6.1.2 pH and Alkalinity

The chemical composition and biodegradability of a substrate are important parameters which can significantly affect the performance of the anaerobic digestion process. Dried coconut copra has a total fat content of 64.5 % in comparison to the 12.6 % fat content of the FVW substrate included in the investigations by Qamaruz-Zaman (2010). High lipid levels in substrates have been known to cause problems for both mesophilic and thermophilic digestion as lipids are easily degraded to LCFAs and VFAs which if allowed to accumulate become toxic to methanogenesis (Dinsdale et al. 1996).The excess accumulation of organic acids can also result in unfavourable decreases of pH levels for methane production as methanogens are more susceptible to pH variation than other micro-organisms in the microbial community (Khanal 2008).

During the batch tests the OLRs which exceeded 15 g VS (Tests 1-6) not only failed to produce significant methane yields but a substantial decrease in pH from an average initial sludge pH of 7.58 to a concluding sludge pH of 5.33-6.40 was also observed (Table 6.2). These results are comparable to investigations by Cirne et al. (2007) of methane production from lipid rich substrates consisting of predominantly whey. For higher amounts of lipids including 31%, 40% 47% (wet weight COD basis) a stronger inhibition was observed in comparison to lower percentages of 5 %, 10% and 18%. In addition, the samples consisting of a lipid content of 47% were reduced to pH 5.7 after only 5 days of the incubation period even though bicarbonate supplements had been added.

Figure 6.6 summarizes both the pH values and alkalinity concentrations from sludge at the conclusion of each test. As illustrated, ORLs exceeding 15 g VS were reported to have effluent pH values outside the optimal range for methanogenic activity (shown shaded). There was also an observed decreasing trend of concluding alkalinity concentrations with the increase of OLRs which could be attributed to a greater consumption of alkalinity from the accumulation of organic acids as the syntrophic relationship between the acetogens and methanogens became imbalanced.

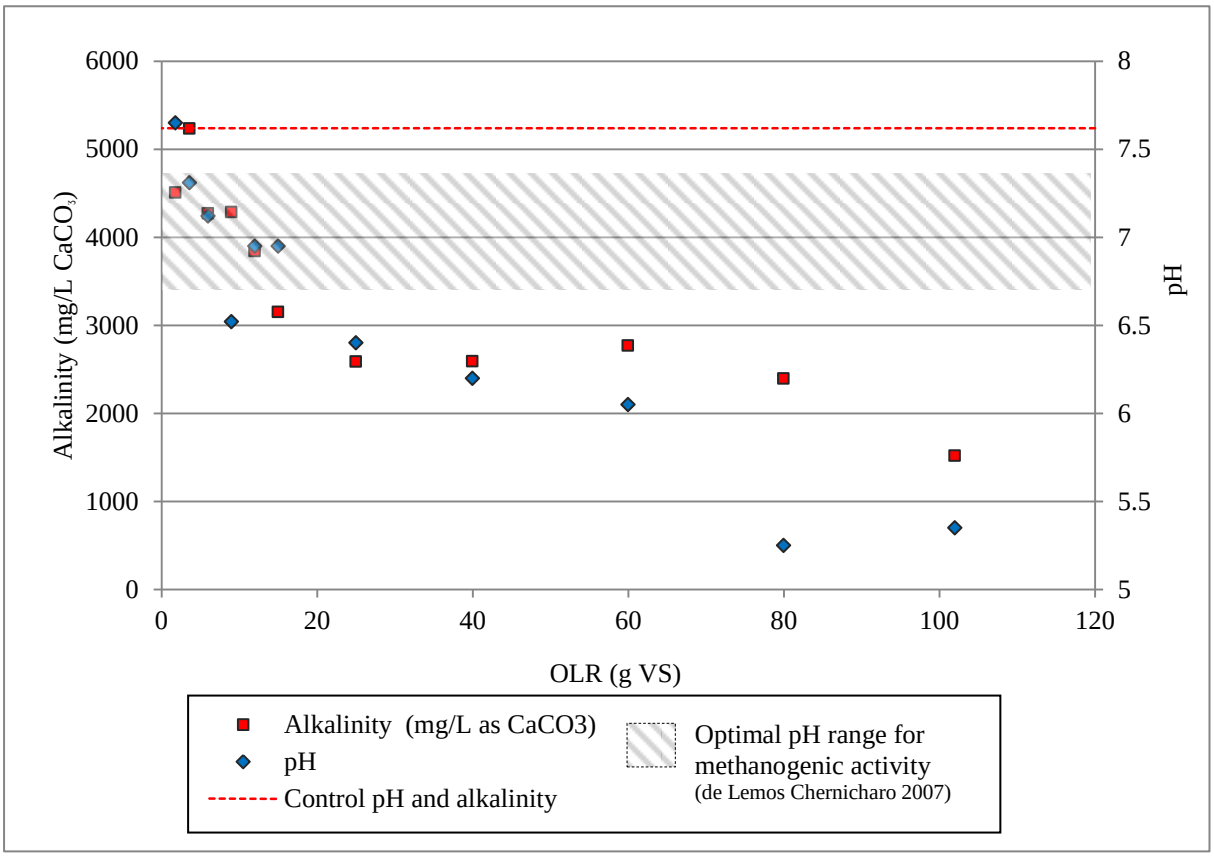

Figure 6.6: Alkalinity and pH at conclusion of Tests 1-14

The inhibition of anaerobic digestion experienced at higher OLRs could be therefore attributed to the overall high fat content and, in particular, the number of LCFAs present in the copra substrate. LCFAs consist of fatty acids with aliphatic tails longer than 12 carbons and collectively equate to approximately 30% of the total VS of coconut copra. The primary LCFAs that have been indentified in coconut copra are myristic ($C_{14}H_{28}O_2$), palmitic ($C_{16}H_{32}O_2$) and stearic acids ($C_{18}H_{36}O_2$) (Appendix B). The efficient hydrolysis of LCFAs is important process to increasing the bioavailability of complex substrates for the use by anaerobic bacteria. However, the degradation of LCFA which takes place through the β-oxidation pathway, has been reported as the rate-limiting step of the whole anaerobic digestion process (Palatsi et al. 2009). LCFAs inhibition in anaerobic digestion has been shown to be related to the physical adsorption of LCFA which can hinder its transfer through

microbial cell walls and have been reported to have an acute toxic effect on anaerobic microbial activity, in some cases in a permanent way (Cirne et al. 2007; Palatsi et al. 2009).

In the literature, the ability to degrade LCFA has been related to microorganisms of the Syntrophomonadaceae and Syntrophaceae families and presently there are only 7 species of syntrophic bacteria reported as capable of growing on fatty acids with more than 12 carbon atoms (Cavaleiro et al.). However, usually a low number of these bacteria are detected in bioreactors treating high LCFAs concentrations due to their slow growth rates (Palatsi et al. 2010). Palastsi et al. (2010) performed batch tests on characterized slaughterhouse waste mixtures with a lipid content of 68-82 % (VS). It was reported that the slaughterhouse waste showed high anaerobic biodegradability and methane potentials however the lipids had a limiting effect on the process kinetics (Palatsi et al. 2010).

6.1.3 Volatile Solids Reduction

Although the main research objective was methane production, the VS destruction percentages at the conclusion of Tests 10 and 13 of 60.4 and 77.1 %, respectively, (Table 6.3) are comparable to similar FVW substrates. Dinsdale et al. (1996) observed a 58% reduction in VS operating batch reactors at a mesophilic temperature with a coffee waste substrate while Zhang et al. (2007) reported VS destruction of 81% digesting FVWs at higher thermophilic temperatures (Dinsdale et al. 1996; Zhang et al. 2007).

Table 6.3: SCOD, Volatile Solids, Volatile Suspended Solids of sludge at conclusion of Tests 1, 9, 10, 13 and 14

Test number	OLR (g VS)	Biogas yield (L)	SCOD (mg/L)	Average biogas composition $CH_4 : CO_2$	VSS:VS ratio	VS destruction (%)
1	102	8.30	15,320	1: 3.87	0.662	45.3
9	9	1.10	10,500	1 : 1.25	0.892	44.5
10	6	3.50	5,075	1 : 0.41	0.626	60.4
13	3.6	3.70	1,275	1 : 0.64	0.806	77.1
14	1.8	1.10	1,700	1 : 1.40	0.692	28.4

However, at the higher OLRs the destruction of VS failed to correlate directly to high methane yields even when large volumes of biogas were produced. This was most clearly demonstrated at the OLR of 102 g VS (Test 1) which produced 8.30 L of biogas with 45.3% VS destruction but achieved a relatively high biogas composition CH_4:CO_2 ratio of 1:3.87 in comparison to the value of 1:0.41 observed at an OLR of 6 g VS (Table 6.3). In addition, at the higher OLR there was an observed decrease from a relatively neutral pH of the initial sludge inoculum to the concluding sludge value of pH 5.33 (Table 6.2).

Investigations by Siegert and Banks (2005) reported that the presence of increasing concentrations of organic acids in a batch anaerobic reactors have shown to have a differential effect on the metabolically distinct phases of hydrolysis, acidogenesis and biogas production associated with the anaerobic digestion process. It was observed that digestion of glucose at different VFA concentrations led mainly to the production of carbon dioxide and a change in the CH_4:CO_2 ratio. The composition of the biogas was also found to change in the cellulose fed digester with the CH_4:CO_2 ratio changing from 1:1.30 to 1:1.90 with an increase in VFA concentration from 1,000 to 8,000 mg/L (Siegert and Banks 2005).

In addition to VS testing, sludge samples at the start and conclusion of Tests 1, 9, 10, 13 and 14 were tested for Volatile Suspended Solids (VSS) concentrations using Standard Methods (A.P.H.A 1998). The VSS/VS ratio sludge values at the conclusion of these tests were reasonably high between 0.626 - 0.892 (Table 6.3) which indicates that a large portion of the VS had remained in the particulate form. The combination of low SCOD concentrations and high VS destruction observed in Tests 10 and 13 also suggests most of the VS destruction was therefore obtained from the solubilised fraction of the substrate. The high VS destruction may also be attributed to difficulties in obtaining homogenous samples from within the reactor as the coconut was extremely buoyant and tended to form floating aggregates.

The presence of a high proportion of the VS in a suspended form may be attributed to poor solubilisation of the lipid fraction in the coconut copra. Liquefaction of lipids has also been found to be rate-limiting for similar high fat substrates and the hydrolysis of complex particulates. Cirne et al. (2007) added a commercial lipase to batch reactors in order to determine whether the enzymatic hydrolysis of lipids was rate limiting for the anaerobic digestion of a whey based substrate. There was a significant difference in the inhibition of methane production reported between the tests that included the additional enzyme and controls samples indicating that the addition of lipase enhanced the hydrolysis of lipids (Cirne et al. 2007).

6.2 Phase II: OLR and Methane Production

As previously described in Section 4.2, the initial start-up load for Run 1 was determined from 20 % of the average of the optimal OLR range achieved from the batch reactors of 3.6-6 g VS (2.4 – 4 g VS/L Rctr). It was noted that throughout each run there were similar general trends for pH, alkalinity and methane production between both Rctr 1 and 2 which indicated a good correlation between the two separate systems (Appendix E). For clarity and illustration purposes, the values achieved with Rctr 2 have therefore been used as a representation of both systems in all figures unless stated otherwise

6.2.1 Run 1: Preliminary Run Testing HRT/SRT

At the initial stages of Run 1 there was a rapid increase in methane percent observed and a maximum of 56.4 % of the total gas was produced within the first 7 days. However, the low overall methane yield coupled with decrease in influent TS concentration of 17,020 mg/L to an effluent concentration of 4,284 mg/L suggested biomass washout was occurring. This may be attributed to the high lipid composition of the coconut substrate as the absorption of a light layer of LCFAs to biomass has been known to lead to the flotation of sludge in continuous systems and subsequent sludge washout (Chen et al. 2008).

Other investigations into the digestion of similar high fat substrates (using a range of different reactor configurations) have also reported system failures due to sludge washout (Hawkes et al. 1995; Vidal et al. 2000). For example, Hawkes et al. (1995) observed poor biomass retention in four different reactors types with an ice-cream wastewater substrate which contained a high proportion of palm oil. Similarly, Vidal et al. (2000) reported that high COD loadings caused problems with sludge flotation which limited the maximum OLR

that could be achieved with the digestion of dairy wastes. In addition, CSTRs without solids separation and recycling are often prone to failure due to excessive biomass washout unless long HRT's (or SRTs) are maintained (Khanal 2008). The process failure of Run 1 was therefore attributed to issues of fluid dynamics rather than inhibition to the microbial population.

6.2.2 Run 2: Secondary Run and Adjustment of HRT/SRT

Due to the biomass washout experienced in Run 1 the HRT/SRT was subsequently extended to 200 days for Run 2. It is possible that the systems could have operated with shorter HRT/SRTs if an external settling tank had been integrated into the process design to allow solids to be concentrated and recycled back into the primary reactor. However, the HRT/SRT remained coupled as the primary research objective was to maximise methane production with a simple operational system and therefore little attention was paid to having large wastage rates.

At the initial stages of the start-up period of Run 2 there was a rapid increase of methane production from a value of 0.261 L CH_4/g VS·day which was observed at 7 days to a maximum 1.29 L CH_4/g VS·day after 14 days. This acceleration in methane production also coincided with a decrease in pH indicating an increase in acidogenic activity (Figure 6.7). Immediately after an increase to an OLR of 24 g VS/day it was observed there was a rapid decrease in pH followed by an increase to normal operating conditions over the next 4-5 days. This trend continued at each new OLR increment however the pH recovery after the increase to an OLR of 48 g VS/day took a considerably longer period of time to re-stabilize (Figure 6.7).

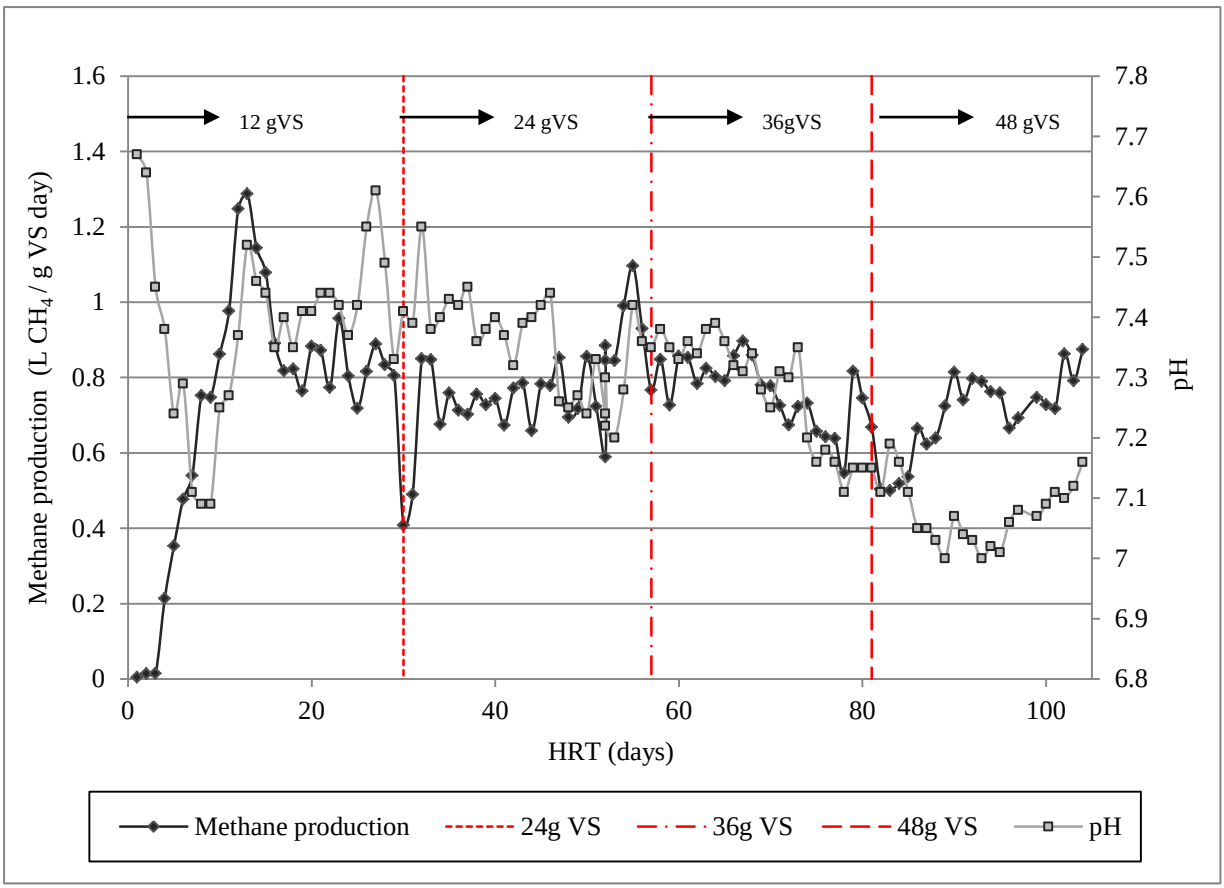

Figure 6.7: Methane production and pH, Run 2

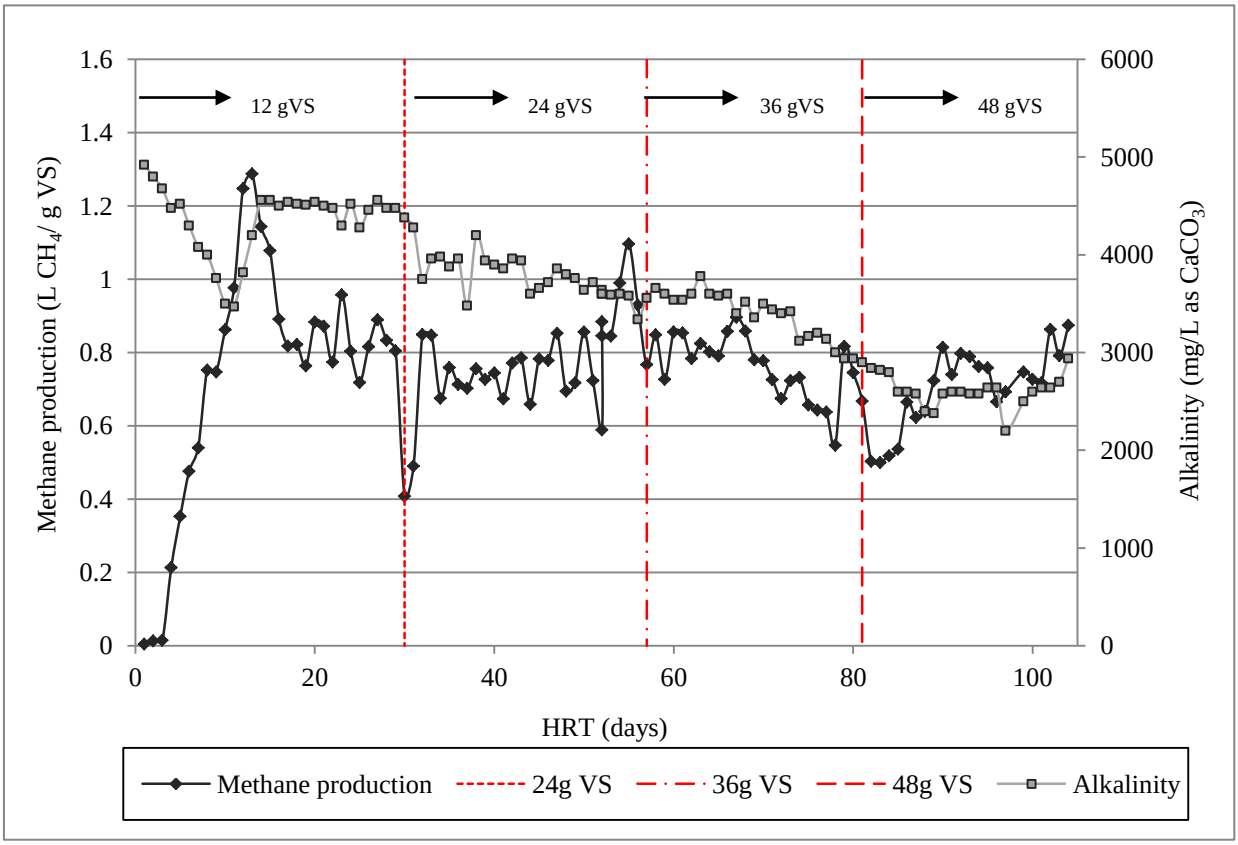

Figure 6.8: Methane production and alkalinity, Run 2

This suggests that there was a decrease in reactor stability at the higher OLR of 48 g VS/day as the reactor was increased towards an optimal OLR and ultimately the limit of the system's capabilities. Qamaruz-Zaman (2010) reported that decreasing pH values and methane percentages were generally an accurate indication at signifying the reactor was becoming acidic and the methane production was ceasing. This was also demonstrated in the batch reactors where a decrease in pH below pH 6.5 in Tests 1-6 coincided with low methane production while reactors with final sludge pH values of 7.10 produced considerably more methane per gram of VS (Table 6.1).

The specific methane production was maximised during Run 2 at an OLR of 24 g VS/day (Table 6.4). The overall average methane production achieved during Run 2 of 0.708 L CH_4/g VS·day (0.628 m^3 CH_4/g VS·day at STP) is also comparable to values achieved with similar mixed FVWs. An average yield of 0.55 m^3 CH_4/g VS was obtained by Viswanth et al. (1992) with a mixed FVW substrate while Mata-Alvarez (1992b) achieved a maximum of 0.480 m^3 CH_4/g VS with organic wastes collected from fruit processing factories.

Table 6.4: Mean methane production, pH and alkalinity, Run 2

OLR (gVS/day)	**Methane production (L CH_4 / g VS·day)**		**pH**		**Alkalinity (mg/L as $CaCO_3$)**	
	Mean	± Stdev	Mean	± Stdev	Mean	± Stdev
12	0.690	0.032	7.39	0.02	4,462	75
24	0.745	0.031	7.38	0.20	3,904	62
36	0.700	0.045	7.31	0.13	3,735	241
48	0.679	0.001	7.20	0.08	3,364	464
Mean	0.708	0.020	7.32	0.03	3,867	188

A mean value for the methane percentage achieved during Run 2 of 67.5 % is also close to the values achieved with other high lipid substrates. For example, Dinsdale et al. (1996) reported methane percentages of 60-65% digesting coffee wastes at thermophilic temperatures while Fountoulakis et al. (2008) achieved percentages of 62-69% with a combination of olive mill, wine grape residue and slaughter house wastewater at a mesophilic temperature (Fountoulakis et al. 2008). A more detailed analysis of methane production observed at different OLRs over a 24 hour period will be discussed in Section 6.3.

The pH values were observed to fluctuate daily however these values remained well above neutral up until an OLR increase to 48 g VS/day (Figure 6.7). The rapid decrease of pH at the initial stages of Run 2 could be attributed to an initial burst of VFA production which is often experienced in the first days of incubation (Qamaruz-Zaman 2010). The pH values were observed to be at the lowest at the OLR of 48g VS/day however this parameter continued to remain within the recommended healthy operating pH range of 6.8 -7.4 for methanogenic activity for CFTRs. There was also an observed decreasing trend in the alkalinity with increase in the OLR although the alkalinity concentrations remained within the recommended ranges of 1,000-5,000 mg/L as $CaCO_3$ found in healthy and typical anaerobic digesters (Figure 6.8) (Grady et al. 1999; Qamaruz-Zaman 2010).

Another indication of reactor stability and performance is the removal of SCOD from the system as the presence of excess amounts in the effluent indicates that there are still large quantities of organic matter not being converted into biogas. Investigations by Qamaruz-Zahman (2010) reported effluent SCOD concentrations below 10,000 mg/L to be an indicator of a healthy reactor system while concentrations over 20,000 mg/L would result in reactor failure. This range of SCOD concentrations is similar to the healthy operating threshold levels observed in the CSTRs during Phase II.

Run 2 remained under the 10,000 mg/L limit recommended for a healthy reactor however as the OLR increased there was a corresponding increase in the SCOD concentrations indicating a steady rise towards the threshold SCOD operating level of 20,000 mg/L (Figure 6.8). The alkalinity remained well within the recommended healthy range of 1,000-5,000 mg/L as $CaCO_3$ and the pH levels within the optimal range for methanogenic bacteria of 6.6-7.4 while the observed trends suggested the rates of decrease of these parameters were not as significant as the increasing concentrations of SCOD (Figure 6.9 and Figure 6.10).

Due to experimental disruption from the 2010 Darfield Earthquake, Run 2 was concluded after 104 days, however, based on a SCOD operating limit of 20,000 mg/L, a theoretical maximum OLR can be extrapolated from the relationship determined between the OLR and SCOD concentrations observed from Run 2 (Figure 6.9). The maximum theoretical OLR hypothesised for a CSTRs using the copra substrate is approximately 125 g VS/day (6.25 g VS / L Rctr·day) which is close to the maximum range of 3.6 - 4 g VS/L Rctr already determined with the batch reactors in Phase I.

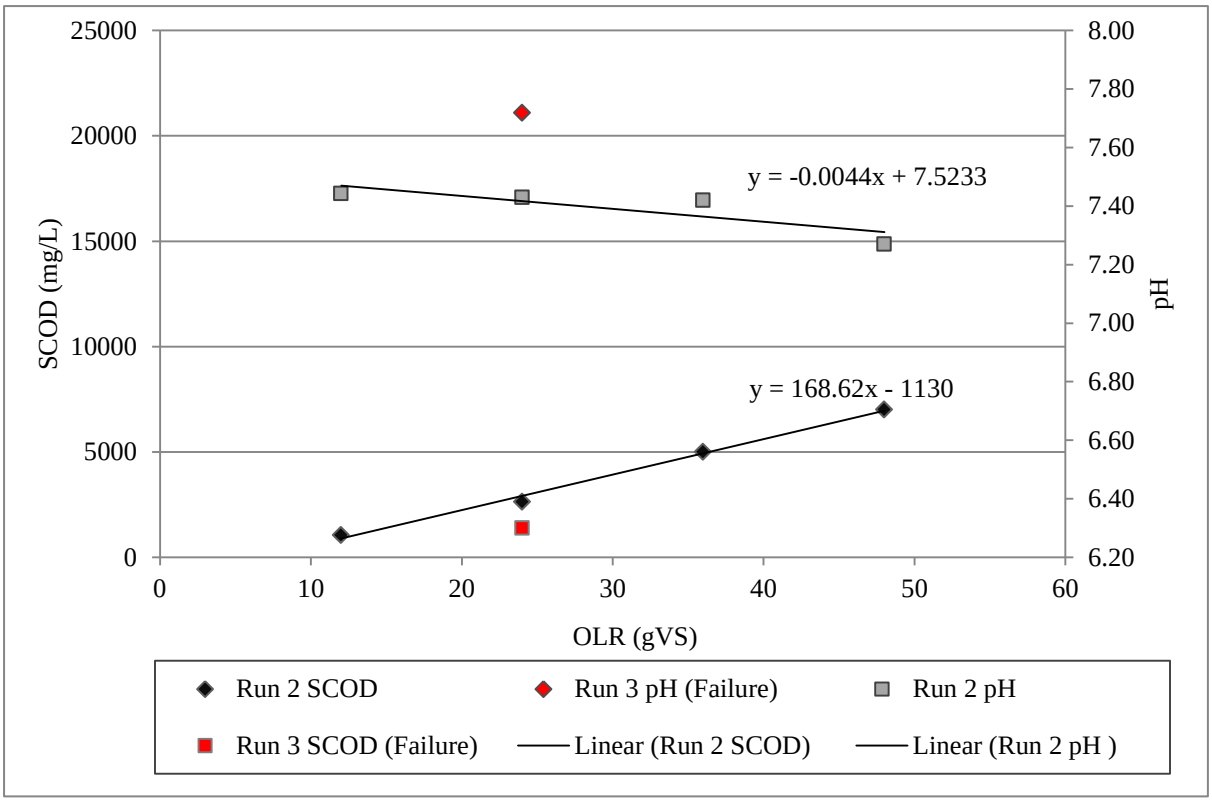

Figure 6.9: Relationship between SCOD, pH and OLR
Run 2 (operational) and Run 3 (Failed values shown red)

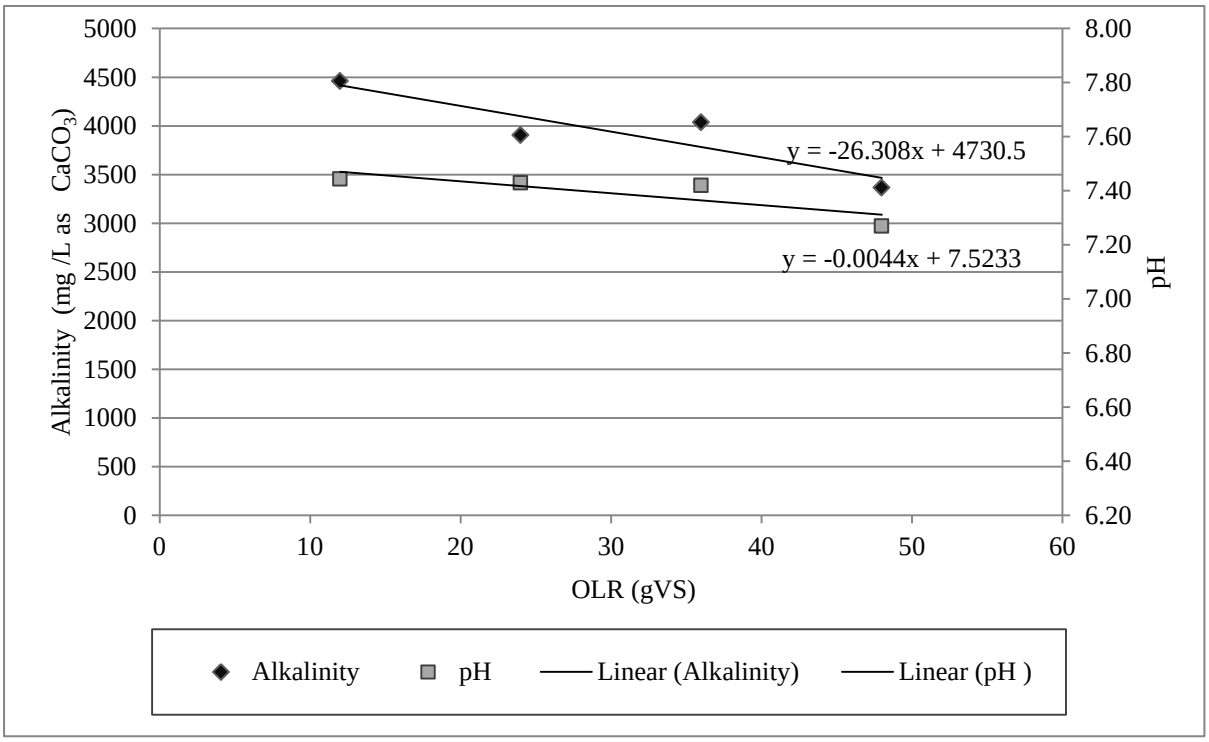

Figure 6.10: Relationship between alkalinity, pH and OLR, Run 2

6.2.3 Run 3: Effects of Accelerated Start-up

In the start-up phase for each reactor, the OLR is increased until the targeted feed loading is achieved since "the micro-organisms in the reactor grow" a balance between the groups of organisms performing several metabolic functions is maintained (Chynoweth 1987). This can ostensibly take long periods of time as the minimum generation time for methanogens is 1-4 days in comparison to acidogens 0.2-0.5 days (IVM 2001). However, if the start-up time could be reduced without negative impacts on the stability of the reactor there would be economic benefits associated with reduced time and materials required before the system is fully operational and able to process influent to the design influent loading.

The initial OLR for Run 3 was identical to Run 2 of 12 g VS/day but was maintained for a HRT of only 4 days before being increased to an OLR of 24 g VS/day. The methane production observed in this time period was slightly higher than Run 2 of 0.174 L CH_4/g VS·day however after peaking on day 6 the methane production rapidly decreased again (Figure 6.11).

In addition, at a HRT of 9 days there was a noticeable increase in the balance gases within the reactor headspace which coincided with a decrease in methane percentage. The rapidly declining pH also indicated that there was an accumulation of acid intermediates occurring from rapid acidogenesis therefore the OLR was decreased back to 12 g VS/day and additional alkalinity in the form of sodium bicarbonate ($NaHCO_3$) was added in order to assist in the recovery of reactor stability. There was slight increase in pH and methane production observed immediately after the alkalinity additions however Run 3 was concluded after 17 days due to negligible gas production and a final pH of 6.30 (Figure 6.11).

In comparison to the previous run, the concluding SCOD concentrations and pH values in the effluent of Run 3 were observed to lie outside the recommend ranges for these parameters

at 21,019 mg/L and 6.30, respectively (Values represented in red, Figure 6.9) However, the alkalinity concentration sampled at the same time remained high at 3,620 mg/L as $CaCO_3$ which can be attributed to the additional $NaHCO_3$ supplements added after a HRT of 9 days.

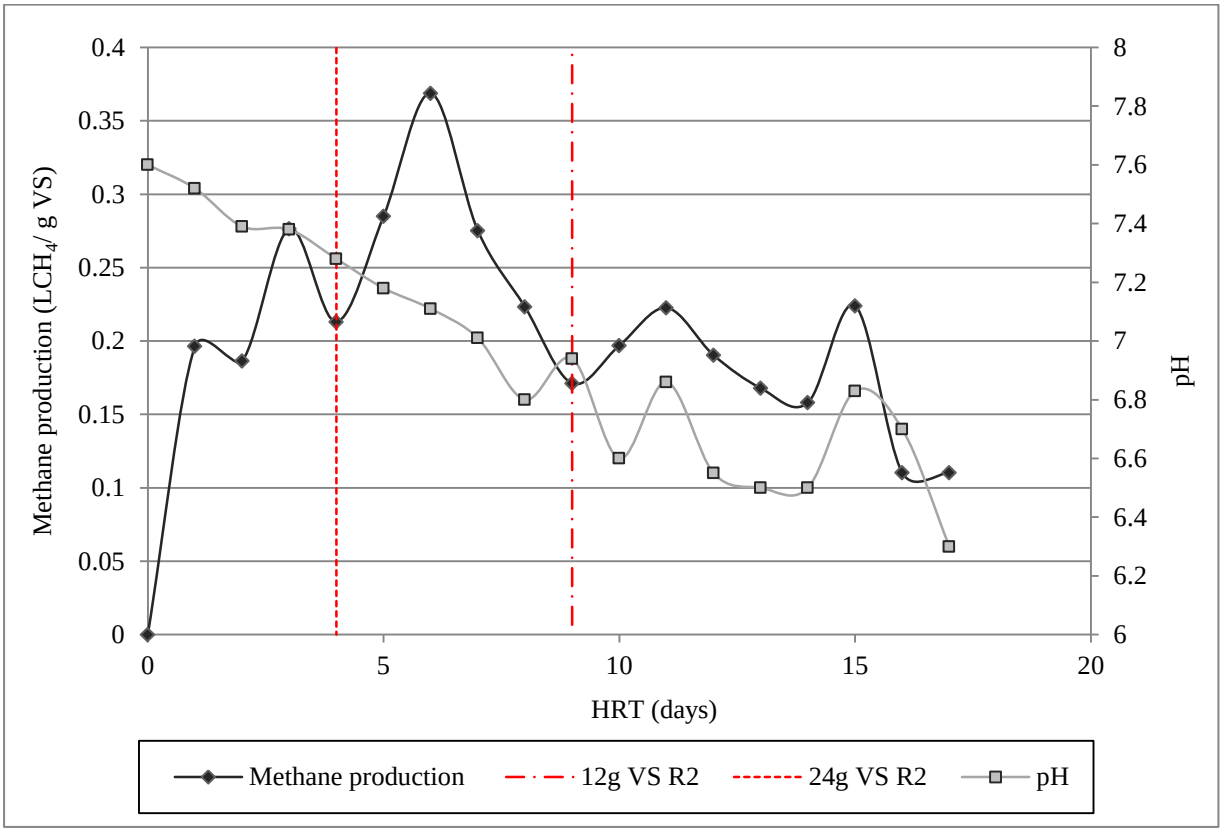

Figure 6.11: pH and methane production, Run 3

6.2.4 Run 4: Effects of Additional Nutrient Supplements

All microbial-mediated processes require nutrient and trace elements during wastes stabilization (Khanal 2008). The primary macronutrients necessary to sustain microbial metabolism are considered to be carbon, nitrogen and phosphorus and the amount required of each is recommended at a ratio of approximately 100-128:4:1 (C:N:P) (Bouallagui et al. 2003). Although the chemical composition of the coconut copra substrate was found to have a C:N:P ratio within this recommended range, additional nitrogen and phosphorus

supplements were added during Run 4 to determine if the methane production could be enhanced.

Various types of both macro and micronutrient supplements can be added in order to stabilize anaerobic reactors and stimulate the anaerobic treatment of a variety of wastes (Kayhanian and Rich 1995). In the literature, diammonium hydrogen phosphate $(NH_4)_2HPO_4$ has been used to successfully supplement nitrogen and phosphorus in the anaerobic digestion of ice cream waste water and mixed FVWs (Hawkes et al. 1995; Lane 1984). The initial concentrations of $(NH_4)_2HPO_4$ to be added daily during Run 4 was estimated at 25 % of the loading rate of 2,000 mg/L used by Lane in the digestion of a similar FVWs (Lane 1984). Diammonium hydrogen phosphate is water soluble therefore it was able to be easily integrated into the daily feeding regime by added it into the coconut slurry mixture before it was poured in the reactors.

The initial methane production for Run 4 was similar to Run 2 and the methane percentages increased to a mean value of 64.4 % within the first 7 days. However, the average methane production rate at the OLR of 12 g VS/day in Run 4 were only 0.320 LCH_4 / g· VS·day in comparison to 0.691 LCH_4/g· VS·day achieved at the same loading rate for Run 2. After a HRT of 25 days, the gas production had declined rapidly and the methane percentage had also decreased to value 45.7% while the CO_2 and balance of other gas had increased to 48.5% and 5.4%, respectively.

The pH had also decreased from an influent pH of 7.45 to an effluent pH of 6.72 after 25 days therefore sodium bicarbonate ($NaHCO_3$) was also added in order to assist in pH stabilization. The addition of $NaHCO_3$ was successful in increasing the effluent alkalinity concentrations of the reactors from 2,800 to 5,340 mg /L as $CaCO_3$ but failed to prevent a

further decrease in pH which declined further to pH 6.42. Run 4 was therefore concluded after 31 days due to low methane production (Figure 6.12).

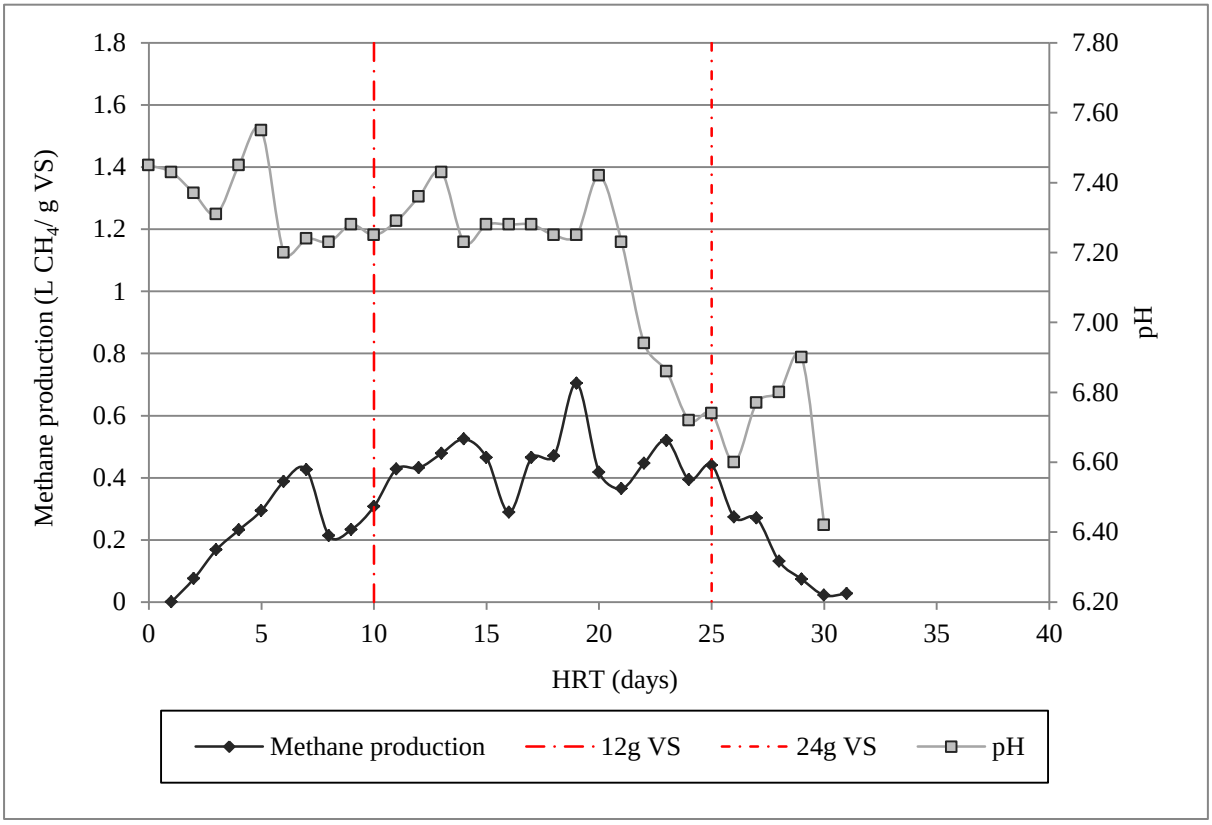

Figure 6.12: Methane production and pH, Run 4

The effluent samples from both reactors were tested for suspected excess ammonia concentrations at the conclusion of the run. Ammonia (NH_3) is one of the hydrolysis products formed during the degradation of proteineous organic materials and continually changes to ammonium (NH_4^+) and vice versa with the relative concentrations of each depending on the temperature and pH (equation 6) (de Lemos Chernicharo 2007).

$$NH^+_4 \leftrightarrow NH_3 + H^+ \quad (6)$$

Ammonia concentrations below 200 mg/L are beneficial to the anaerobic process since nitrogen is an essential nutrient for anaerobic micro-organisms (Chen et al. 2008). Both the

ammonium ion (NH^+_4) and the free ammonia (NH_3) can inhibit the anaerobic process when present in high concentrations however significant differences can be found in the literature regarding the levels at which inhibition occurs. Siles et al. (2010) reported inhibition of methane production with free ammonia (FA) concentrations of 620 mg/L in thermophilic digesters while Hansen et al. (1997) found FA concentrations exceeding 1,100 mg/L caused inhibitions in culture at pH 8.0 (Hansen et al. 1998; Siles et al. 2010). The NH_3-N concentrations sampled at the conclusion of Run 4 for Rctr 1 and Rctr 2 were 964 and 1,125 mg/L NH_3-N, respectively. These levels combined with the low gas production experienced during Run 4, suggest that reactor performance was inhibited by high NH_3 concentrations from the accumulation of excess nitrogen from the $(NH_4)_2HPO_4$ additions.

6.2.5 Run 5: Effects of Mixing Frequency

At the conclusion of Runs 3 and 4, it was noted that there were fatty deposits visible on the surface of the sludge inside both reactors which indicated the fat components of the substrate had not been totally degraded (Figure 6.13 and Figure 6.14). It was also observed that at the conclusion of Run 4 there was a large quantity of whole, undigested copra particles floating within a layer of suspended solids at the top of the active volume.

Figure 6.13: Internal contents at conclusion Run 3

Figure 6.14: Internal contents at conclusion of Run 4

The accumulation of fats, oils and greases (FOGs) can affect the performance of anaerobic digesters and is a well documented problem in the literature (Cirne et al. 2007)) . FOGs, that is together with other floating materials, can form a thick scum layer on the top of the sludge surface which reduces the active volume of the tank and causes problems with operation and system maintenance. For these reasons, complex FOG wastes present in the influent to WWTPs are usually removed during the first steps of treatment in order to avoid subsequent mechanical problems caused by the increase in solid sludge throughout the treatment process (Martín-González et al. 2010).

Mixing is an important consideration for substrates that have high lipid content as it not only increases contact time between micro-organisms and their food but it also helps disrupts the FOG layer that may accumulate on the surface. In addition, mixing may assist in limiting inhibitions caused by LCFAs as reported by Rinzema et al. (1993) who concluded that efficient anaerobic degradation of C_{12} fatty acids required good mixing and substrate contact (Rinzema et al. 1993).

The effect of mixing over a 24 hour period was therefore investigated under three different conditions during Run 5; (1) mixing for 5 min/day prior to sampling and feeding, (2) mixing for 1 minute every 72 min and (3) mixing for 1 min every 15 minutes. Continuous mixing of the CSTRs could not be achieved as the heat generated from the internal stirrers significantly increased the internal temperature of the reactors to above 47°C and outside the desired mesophilic temperature range. It was observed that an increase in the mixing frequency encouraged the biogas to be released from the sludge in rapid surges, which were represented as sharp peaks in methane gas production immediately after the stirrers were turned on either manually or through the automated mixing system (Figure 6.15, Figure 6.16 and Figure 6.17). The average methane production rate was able to be increased up to 5 times more through a more frequent mixing regime from 5 min/day to 1 min/15 min (Table 6.5).

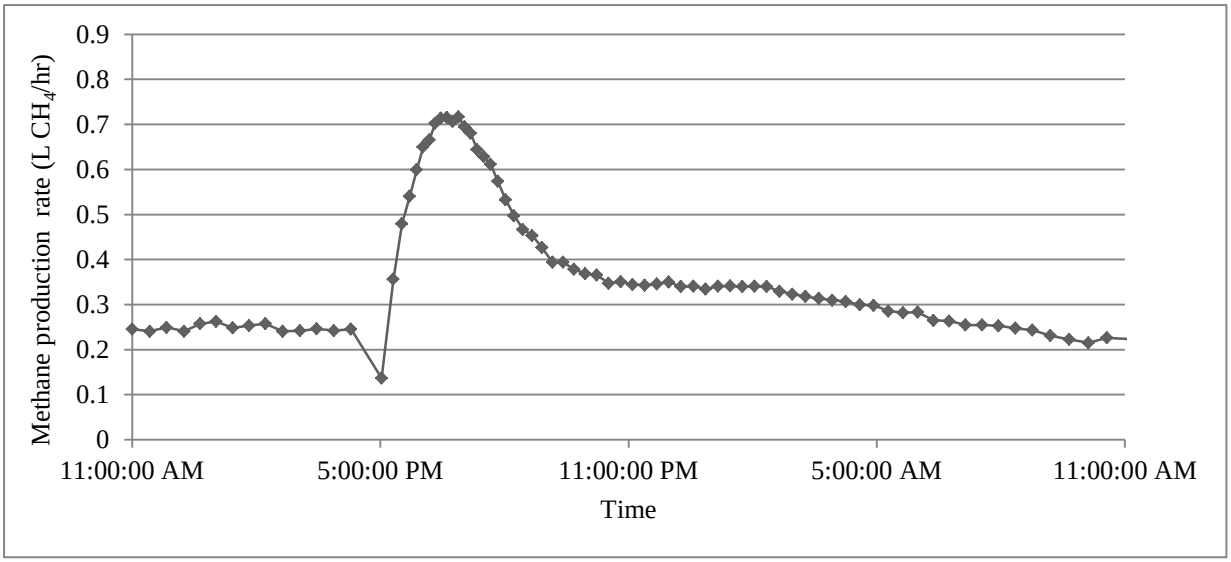

Figure 6.15 : Methane production rate, mixing 5 min/day

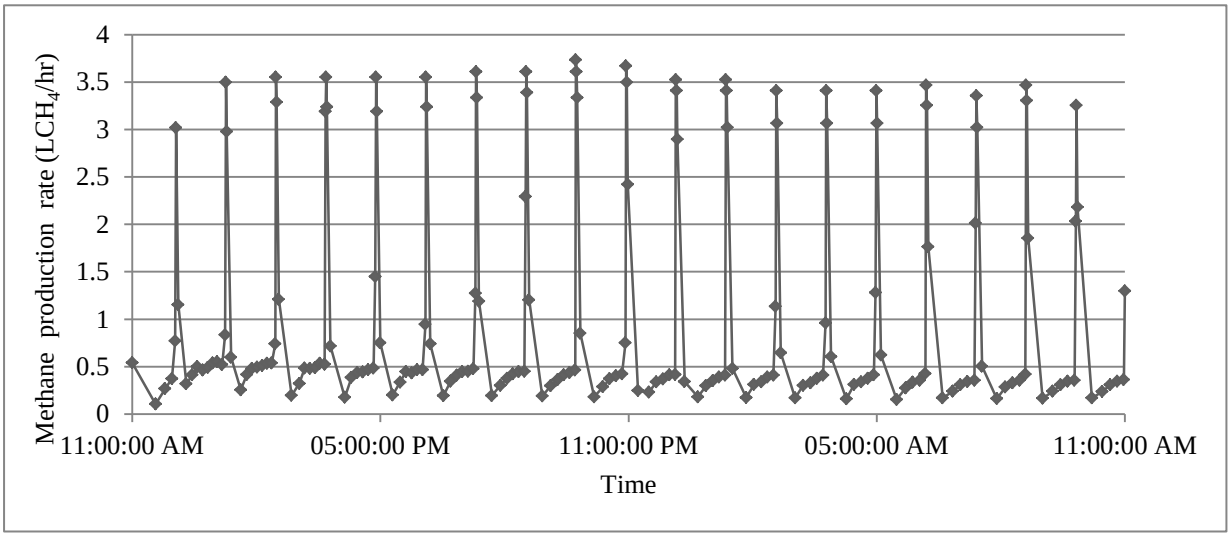

Figure 6.16: Methane production rate, mixing 1min/72 min

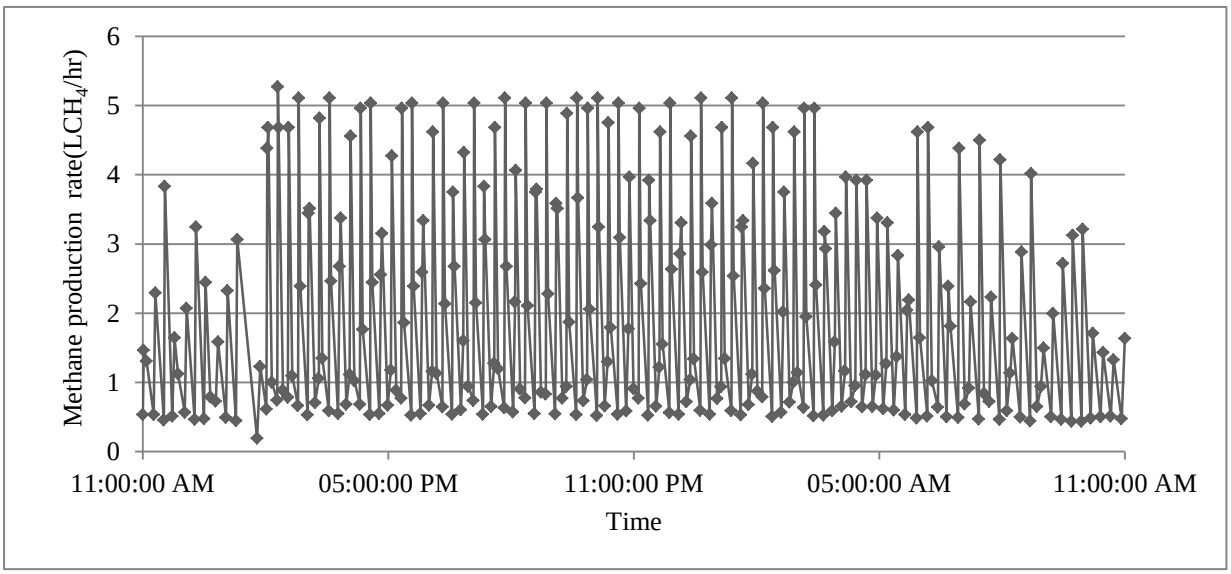

Figure 6.17 : Methane production rate, mixing 1 min/15 min

Table 6.5: Methane production rate with varied mixing regimes, Run 5

Mixing regime	Methane production rate (LCH_4/ hr)		Methane percentage* (%)	
	Mean	±Stdev	Mean	±Stdev
5 min/ day	0.381	0.667	63.3	1.15
24 min/day	1.145	1.230	65.6	3.39
96 min/day	2.078	1.570	68.5	0.64

*Average taken over 7 day period R1 and R2

It has been suggested in the literature that the phase transfer of gaseous products can be rate limiting and that at high substrate concentrations, gas bubbles may surround bacteria and interfere with substrate diffusion (Chynoweth 1987). This was also illustrated in investigations by Finnery et al. (1975) who were able to increase the methane formation rate as much as six fold by employing vigorous agitations (Finney and Evans 1975).

Ultimately anaerobic digestion requires that the substrate come in contact with the bacteria or enzymes required for degradation. An increase in the mixing frequency may therefore have a positive effect on the degradation of the substrate by promoting greater dispersion throughout the bacterial population.

Previous research has also suggested that the process controlling factor with high lipid substrate is the liquefaction of the colloids adsorbed by the bacteria and the hydrolysis of suspended solids entrapped within the biomass (Masse et al. 2003). However, it is difficult to determine in this research whether the higher methane production rate was a result of increased microbial contact with the substrate, the release of gas particles entrapped within the sludge caused by increased turbulence or a combination of both factors. Further studies could potentially include CSTR systems with an external mixing mechanism that would have a reduced impact on the reactors' internal temperature and therefore allow a fully continuous mixing regime to be investigated. However, the excess accumulation of lipids from the

degradation of the coconut copra substrate could potentially result in mechanical operational problems above the quantities tested in the CSTRs during Phase II. This consideration may ultimately restrict the maximum OLR that could be achieved well before inhibition to the microbial population from substrate overloading occurs.

6.3 Comparisons of Methane Production: Phase I and Phase II

The methane production for both Phase I and II were compared directly by applying the equivalent OLR over an incubation period of 8 days to each reactor volume in order to obtain a normalised methane production rate (L CH_4/L Rtr·g VS·day). The methane production rate was represented for both batch reactors and CSTRs at the OLRs of 1.2 g and 2.4 g VS/ L Rctr. Continuous systems typically have higher biogas production than batch digesters and are generally able to tolerate higher OLR (Khanal 2008). However, the average methane production rates for the batch reactor systems were observed to be slightly higher at 0.037 and 0.032 LCH_4/L Rtr·g VS· day at an OLR of 1.2 g and 2.4 g VS/ L, respectively, than the CSTRs which achieved a mean value of 0.030 and 0.026 LCH_4 / L Rctr·g VS· day (Figure 6.18 and Figure 6.19).

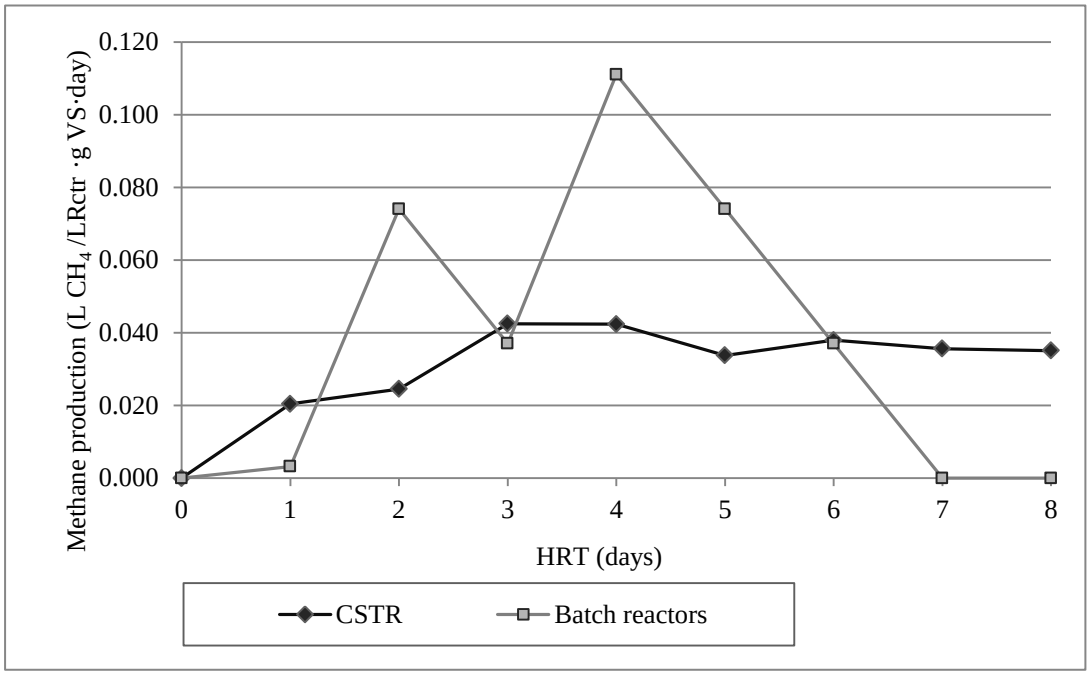

Figure 6.18: Methane production rate, OLR 1.2 g VS/L Rctr

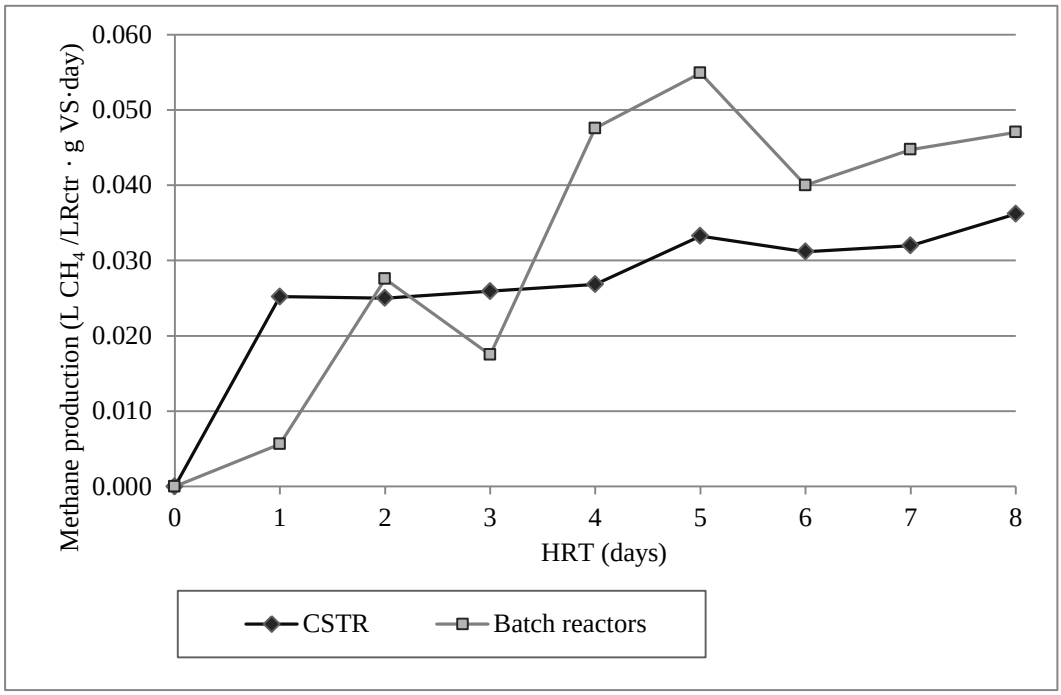

Figure 6.19: Methane production rate, OLR 2.4 g VS/L.Rtr

Although the methane production rate for the CSTRs was less than the batch tests these results are in agreement with research using similar systems. Qamaruz-Zaman (2010) reported the actual methane production in a continuous reactor to be slightly lower at 0.32 L CH_4/g. VS than the estimated value of 0.35-0.40 L CH_4/g. VS which was based on the rate achieved in the batch studies over a 60 day period.

These results suggest there could be some limitations with the scale up of OLRs from batch studies to CSTRs with the coconut copra substrate which could be attributed to the poor solubilisation of the lipid fraction of the coconut copra and/or the accumulation of FOGs at the surface in the CSTRs. However, these problems could be potentially resolved with an increase of mixing which would ensure a greater dispersion of the substrate and help prevent stratification within the reactor.

6.4 Theoretical methane yield

Substrate composition is a major factor affecting both the methane yield and methane production rates in the anaerobic digestion process. Organic matter is the main source of energy for micro-organisms involved in the anaerobic digestion process therefore if the chemical composition of the substrate is known, a theoretical methane production (TMP) can be calculated using Bushwell's equation (equation 7) (Chynoweth 1987)

$$C_nH_aO_bN_c + \left[n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right]H_2O \rightarrow \left[\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right]CH_4 + \left[\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right]CO_2 + cNH_3 \quad (7)$$

By weight lipids will produce typically 1.2 m^3/ kg of biogas in comparison to 0.7 m^3 /kg of protein and 0.8 m^3/ kg of carbohydrates (Gray 2004). Dried copra consists of approximately 65% fat, 23% carbohydrates and 7% protein which are degraded to fatty acids, sugars and amino acids during the hydrolysis stage.

To calculate the TMP, the chemical formula used for the fraction of carbohydrates present in the copra substrate was glucose ($C_6H_{12}O_6$) while for protein a generic formula ($C_4H_{6.1}O_{1.2}N$) was used (Eastman and Ferguson 1981). There are a number of medium and long chained, saturated fats (6-18 carbons) present in coconut copra however lauric acid contributes approximately 49% of the total fat content therefore the chemical formula for lauric acid ($C_{12}H_{24}O_2$) was used to represent the total fat fraction. (Appendix B). By incorporating the equivalent weights and amounts of carbohydrates, protein and fats present in each gram of coconut substrate into Bushwell's equation, the TMP for both the batch reactors and CSTRs at each OLR was calculated. These values were then compared to the actual mean methane production observed for both anaerobic system types and the results were expressed as an efficiency of conversion percentage (Table 6.6, Table 6.7 and Appendix F).

Table 6.6: Batch reactors theoretical methane production (TMP) Tests 1-14

Test	OLR (gVS)	TMP (L/CH_4)	Actual methane production (L/CH_4)	Efficiency (%)
1	102	85.03	1.02	1.2
2	102	85.03	1.15	1.4
3	80	66.70	0.98	1.5
4	60	50.02	0.11	0.2
5	40	33.35	0.29	0.9
6	25	20.84	0.08	3.8
7	15	12.51	0.19	1.5
8	12	10.00	0.16	1.6
9	9	7.50	0.22	3.0
10	6	5.00	1.76	35.2
11	6	5.00	1.10	22.0
12	6	5.00	0.12	2.4
13	3.6	3.00	1.60	53.3
14	1.8	1.50	0.16	10.7

Table 6.7: CSTRs theoretical methane production (TMP) Run 2

OLR (gVS/day)	TMP ($L/CH_4 \cdot day$)	Actual mean methane production ($L/CH_4 \cdot day$)	Efficiency (%)
12	9.75	8.30	83.0
24	19.50	17.89	89.4
36	29.26	25.07	83.5
48	39.01	33.63	84.0

The highest efficiency for the batch reactors was achieved at the OLR of 3.6 g VS (Test 13) where the actual methane yield measured was 53.3% of the TMP. As the OLR was increased during the batch reactors tests, the efficiency percentage decreased and the lowest efficiencies were observed at 60 and 40 g VS (Test 4 and 5) at 0.2 and 0.9 %, respectively (Table 6.6). This could possibly be attributed to substrate overloading of the reactors at the higher OLRs which resulted in pH values outside the optimal range for methanogenic activity and subsequently low methane production.

Although the batch reactors were able to produce higher methane production per reactor volume (L CH_4/ L Rctr·g VS) the CSTRs achieved a greater methane production efficiency. The CSTRs efficiency percentages at all the OLRs during Run 2 were observed to be above 80% with a maximum of 89.4 % achieved at an OLR of 24 g VS/day (Table 6.7). This is probably attributed to the much longer retention times (up to 104 days) that the CSTRs were operated at in comparison to the 8 day incubation period of the batch reactor systems. A long retention time allows the microbial population to acclimate against potentially inhibitory LCFAs, therefore greater efficiency in both degradation and bacterial utilization of the coconut copra substrate is observed. The production of methane and substrate degradation using the anaerobic digestion of similar high lipid wastes has also been reported to have increased with the use of acclimated microbial consortiums (Alves et al 2001; Goncalves 2010). Goncalves et al. (2010) demonstrated that the anaerobic digestion of olive mill wastewater (OMW) which had a high fat content was enhanced by using an adapted consortium to LCFAs. Subsequently, a greater resistance to LCFA toxicity was achieved and the biodegradation rate was improved which enhanced the overall biogas production. In addition, Alves et al (2001) reported that sludge that had been pre-exposed to lipids showed higher tolerance to oleic acid ($C_{18}H_{34}O_2$) toxicity than the sludge that had been fed with a

non-fat substrate. The sludge that had been acclimatized with lipids also showed an increasing biodegradation capacity of oleic acid during the trial (Alves et al. 2001).

6.5 Soluble Carbon Oxygen Demand (SCOD) and Volatile Fatty Acids (VFA) Track Studies

6.5.1 Phase I: VFA and SCOD Concentrations

For the samples taken at the conclusion of each test in the batch reactors, there were one or more peaks that registered on the GC response curve at the pre-determined time intervals for acetic, propionic, butyric and also isovaleric acids. As the OLR increased there were an increasing number of the higher molecular VFAs detected, however, the areas under these peaks revealed that the concentration of VFAs were present in very small quantities (< 5 mg/L) (Figure 6.20 and Figure 6.21).

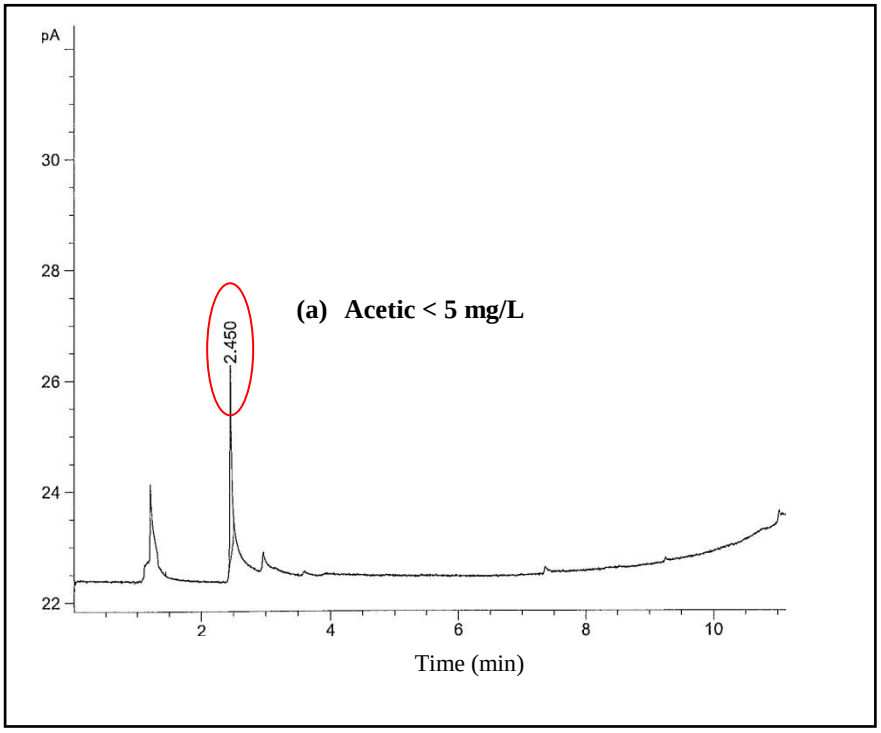

Figure 6.20: GC response curve, 6 g VS

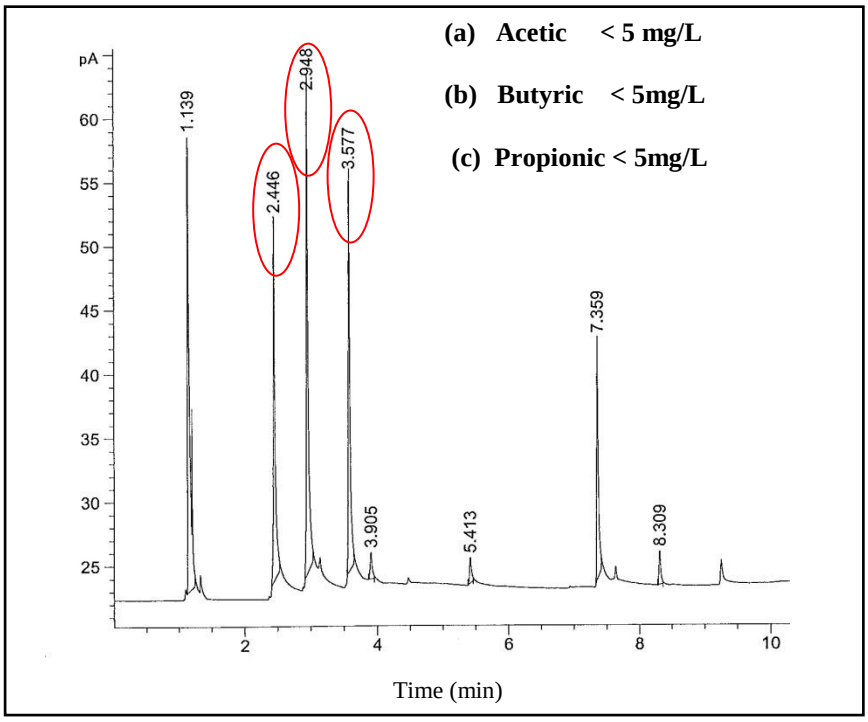

Figure 6.21: GC response curve 80 g VS

There was also an observed increase in the SCOD concentrations with increase in OLR which coincided with a decline in pH values in the samples analysed. At an OLR of 102 g VS (Test 1) the concluding SCOD concentration and pH values were 15,320 mg/L and 5.33, respectively, while in comparison at the OLR of 6 g VS (Test 10) the values for the same parameters were 5,075 mg/L and 7.12 (Table 6.8).

Table 6.8: SCOD and pH values; batch reactors Tests 1,9,10,13 and 14

Test number	OLR (g VS)	SCOD (mg/L)	pH	VFA concentration (mg/L as acetic)	Mean methane yield (L CH_4 / g VS)
1	102	15,320	5.33	< 10	0.010
9	9	10,500	6.52	< 10	0.103
10	6	5,075	7.12	< 10	0.277
13	3.6	1,275	7.31	< 10	0.420
14	1.8	1,700	7.65	< 10	0.084

The low concentrations of VFAs indicates that the decrease in pH was not directly related to an excess accumulation of VFAs and possibly other medium, LCFAs or other factors were contributing to the low pH values and ultimately souring of the batch reactors at the higher OLRs tested. In addition, the low VFA concentrations, neutral pH and high methane production achieved in Tests 10 and 13 of the batch reactors suggest VFAs were utilised shortly after their production, therefore a balance between acidogenesis and methanogenesis was achieved. The low VFA concentrations determined from the GC results indicate that other soluble organics from either the solubilised coconut substrate or other by-products from the anaerobic process, were potentially contributing to the majority of the SCOD present at the conclusion of each test.

The low pH could be attributed to the increasing partial pressure of CO_2 at the higher OLRs of Tests 1-7. Unlike methane gas, CO_2 is very soluble and the transfer rate from the liquid phase within the sludge to the gas phase depends on a number of factors including reactor design, partial pressure of the gas in the headspace and also temperature (Khanal 2008). Large volumes of biogas were produced within the first 3 days of the batch Tests 1-7 however up to 56.6 % of the total biogas was observed as CO_2. In addition, Tests 1-7 also had the lowest pH sludge values at the conclusion of each test. Increasing CO_2 partial pressure observed within reactors as also been reported in the literature as having a depressing effect on the pH value (Vavilin et al. 1995).

6.5.2 Phase II: VFA, SCOD Track Study and Patterns in Methane Production

6.5.2.1 FVFA Track Study

Similar to the batch studies, the area under the peaks in the GC response curves for the samples analysed in the CSTRs track studies indicated the concentrations of VFAs were present in very small quantities (< 5 mg/L) (Table 6.9) However, there was a trend of increasing concentrations of VFAs, especially the higher molecular VFAs, observed throughout the time period immediately after feeding (Figure 6.22). There was also an increase in the VFA concentrations observed in the CSTR track studies at an OLR 48 g VS/day in comparison to an OLR of 12 g VS/day (Figure 6.23).

Table 6.9: Comparisons of methane production rate over 24 hr period at varying OLRs

OLR/day (g VS/day)	**Methane production rate (L CH_4 / hr)**		**Methane production rate / OLR (L CH_4 / hr·g VS)**		**VFA concentration (mg/L as acetic)**	**Percentage of total methane achieved in first 12 hours (%)**
	Mean	± Stdev	Mean	± Stdev		
12	0.482	0.095	0.040	0.008	< 10	57.8
24	2.028	0.512	0.084	0.019	< 10	49.4
36	2.092	0.487	0.058	0.014	< 10	46.5
48	2.889	0.478	0.060	0.001	< 10	42.7

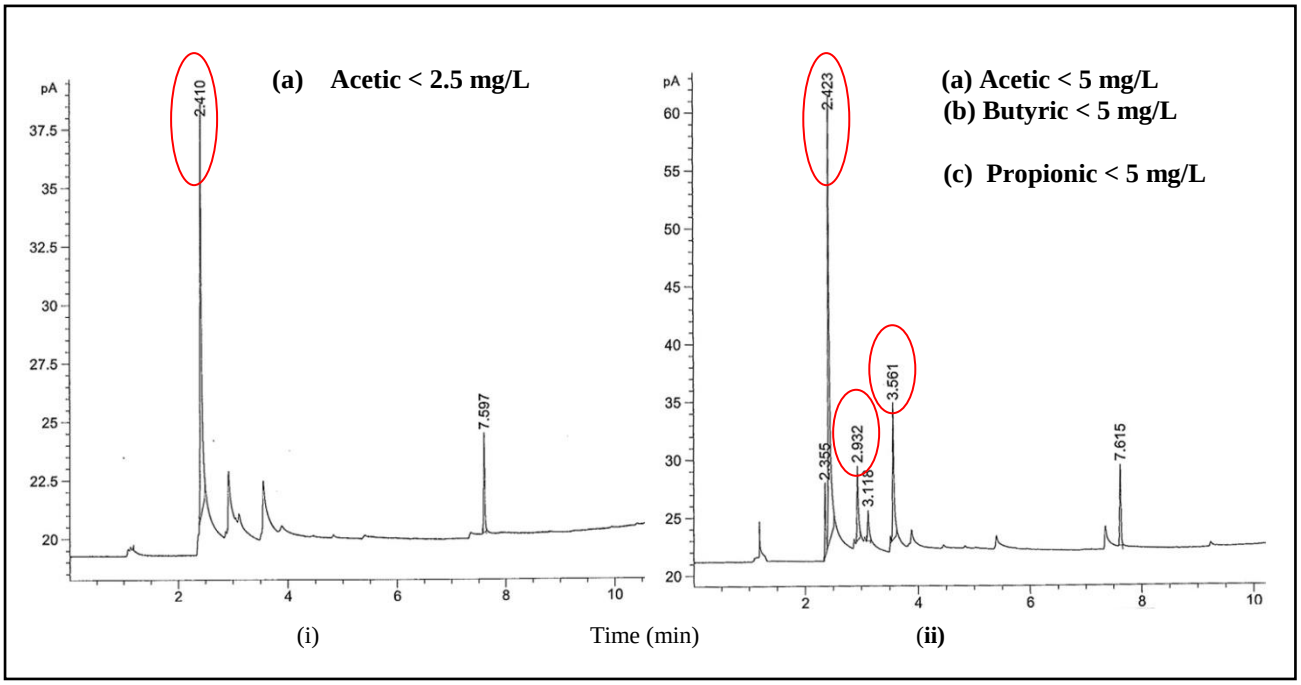

Figure 6.22: GC response curve, 48 gVS/day (i) 30 mins after feeding (ii) 3 hrs after feeding

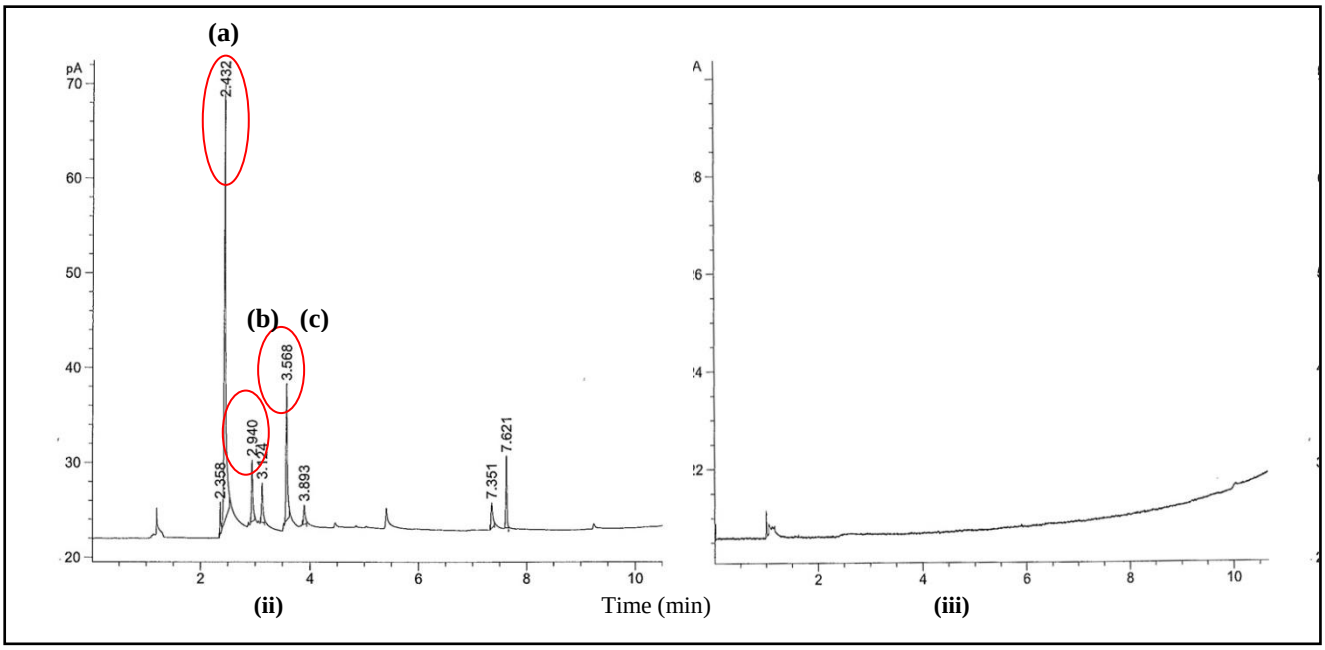

Figure 6.23: GC response curves (ii) 48 g VS/day (refer Figure 6.22 for descriptions) (iii) 12 g VS/day

These results suggest that a portion of the coconut substrate was being hydrolysed and converted to VFAs through acidogenic activity and the concentration of these acids was gradually accumulating as the OLR increased (Figure 6.23). The concentrations of the VFAs detected however remained well below levels toxic to methanogenic activity at all OLRs tested in the CSTRs studies. The low VFA concentrations and high gas production observed during the track studies also suggest that the acid intermediates were being utilized by methanogenic bacteria at a rapid rate.

6.5.2.2 SCOD Track Study and Patterns in Methane Production

At an OLR of 12 g VS/day, the average methane production rate (L CH_4/ hr·gVS) was less than half of the OLR of 24 g VS/day (Table 6.9). The quantity of substrate available at 12 g VS/day therefore appeared to be the limiting factor in the anaerobic process as the methane production remained constant at a mean value of 0.482 L CH_4/ hr·gVS, with an absence of sharp peaks which indicates the substrate was both hydrolyzed and the intermediates converted to methane at a constant rate (Figure 6.24). This was confirmed by the very low SCOD (<500 mg/L) concentration tested in the effluent 4 hours after feeding and the relatively neutral pH which remained within the range of pH 7.20 and 7.55 (Figure 6.25).

When the OLR was doubled to 24 g VS/day the gas production became less uniform and a distinct peak in methane production was observed 3 hours after feeding (Figure 6.24). There was also a corresponding decrease in SCOD concentration observed in the reactor effluent from 2,900 mg/L approximately 2 hours after feeding (13:00) to 1,340 mg/L 4 hours after feeding (15:00) while the pH remained relatively neutral and within the range of pH 7.28-7.46 (Figure 6.26). Although the overall biogas production was slightly lower at 3.9 L/ hr (2.8

L CH_4/hr) this particular pattern is also consistent with gas production patterns reported over a 24 hour period by Viswanath et al. (1992). During investigations digesting a mixed FVW substrate the maximum biogas rate of approximately 5.1 L/ hr was reported at the fourth hour after feeding (Viswanath et al. 1992).

As the OLR was increased again to 36 g VS/day the methane production did not peak until 9 hours after feeding (Figure 6.24). The SCOD concentration remained within a range of 2,520–3,180 mg/L and the pH remained neutral with few fluctuations during the 4 hours immediately after feeding (Figure 6.27). At the OLR of 48 g VS/day a similar surge in methane production was observed at approximately the same time period as an OLR of 36 g VS/day (Figure 6.24). However, the methane production rate and SCOD also remained high even before feeding indicating there were potentially residual acid intermediates still present from the previous feeding which were still being utilized by methanogenic bacteria.

This is a similar observation to tracking studies reported by Checci et al. (2007) in the anaerobic digestion of the organic fraction of FVWs. It was reported that the OLRs of 2.1,3.2 and 4.2 g VS/m^3·day showed noticeable decreasing methane production rates after experiencing a peak rate immediately after feeding however at a higher OLR of 6.9 kg TVS/m^3·day, the gas production rate remained constant until the next feed. It was also observed the more than 60% of the gas was produced within the first 12 hours of anaerobic digestion after feeding the reactors the FVW substrate (Cirne et al. 2007).

Similar to these investigations by Checci et al. (2007), at the OLR of 12 g VS/day of the coconut substrate, approximately 58 % of the methane production occurred within the first 12 hours immediately after feeding. As the OLR was increased there was a noticeable lag period between the feeding time and peak methane production and a subsequent decrease in the

percentage of methane produced in the first 12 hour of the 24 hour period (Table 6.9). This suggests that with an increase in OLR there is a corresponding delay in methane production potentially from inhibitions from the LCFAs present from the degradation of the lipids in the coconut substrate. Beccari et al. (1999) also investigated the inhibitory effect of LCFAs, in the form of oleic acid on methanogenesis. It was reported that strong inhibition and delays in methane production were experienced and that the methanogenesis lag phase was doubled with the addition of oleic acid to olive mill wastewater. Similarly, Alves et al (2001) concluded that when oleate was added to anaerobic fixed–bed units, a lag phase of variable duration (depending on the concentration of oleate) was observed in the degradation of the substrate.

At the OLR of 48 g VS/day the SCOD concentrations observed were considerably higher and more erratic in trend than at lower OLRs (Figure 6.28). However, the methane production rate remained similar to the previous OLRs and pH values remained relatively neutral indicating the reactor was still within the ranges of healthy operating parameters for anaerobic digestion (Table 6.9 and Figure 6.28).

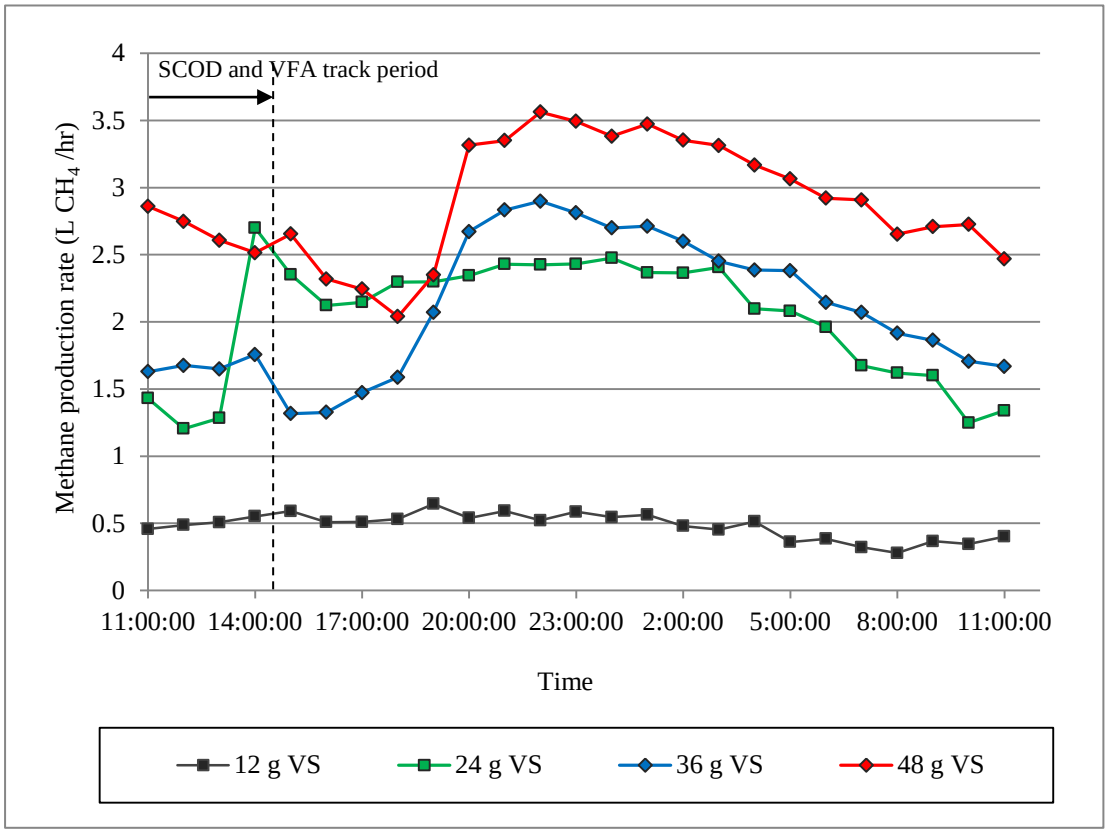

Figure 6.24: Methane production rates 24 hours after feeding, Run 2

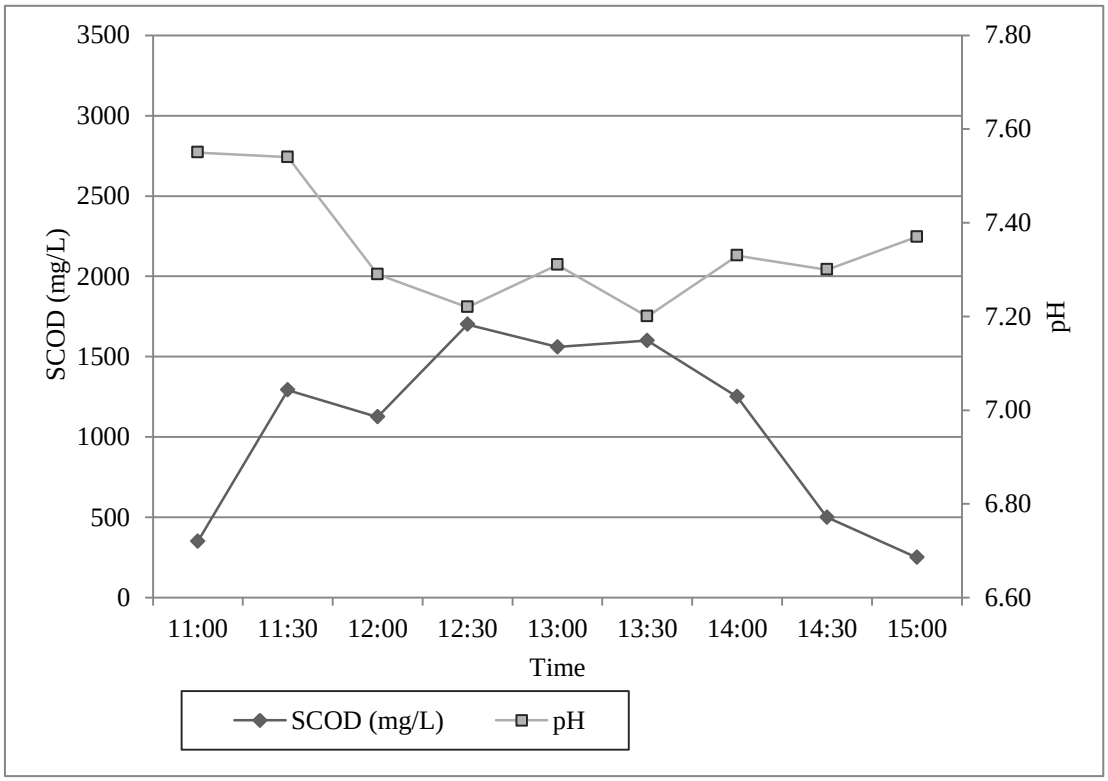

Figure 6.25: SCOD and pH tracking study, 12g VS/day

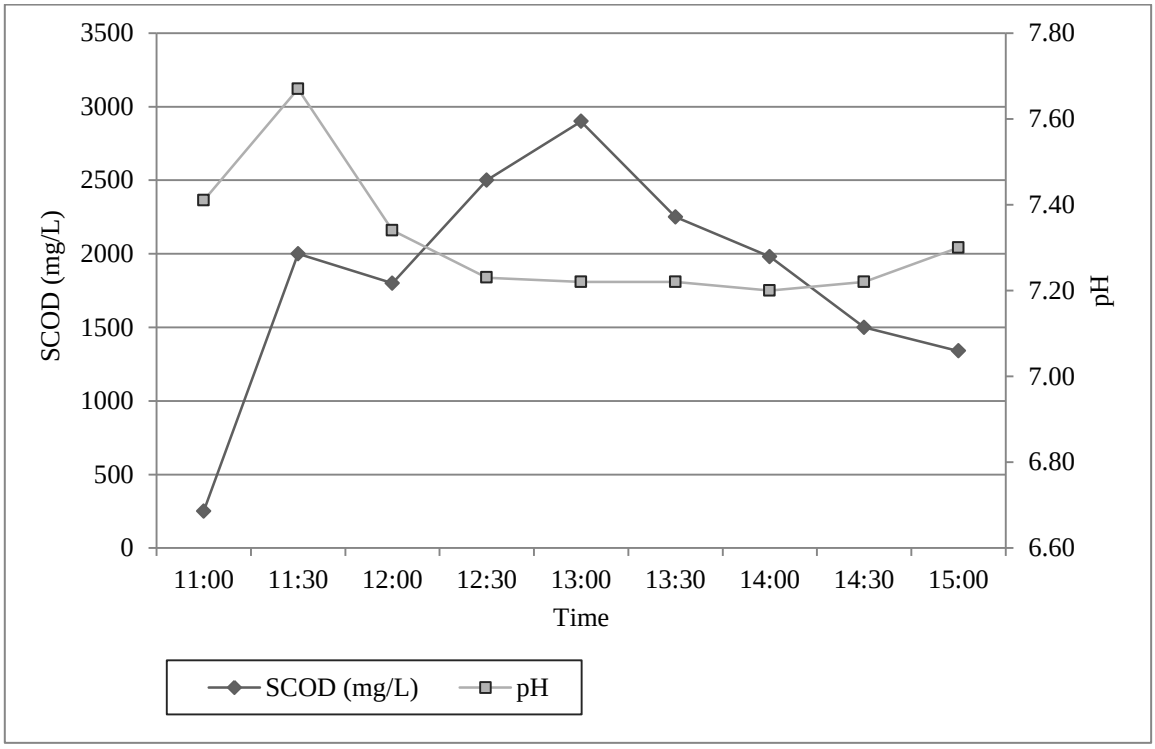

Figure 6.26: SCOD and pH tracking study, 24g VS/day

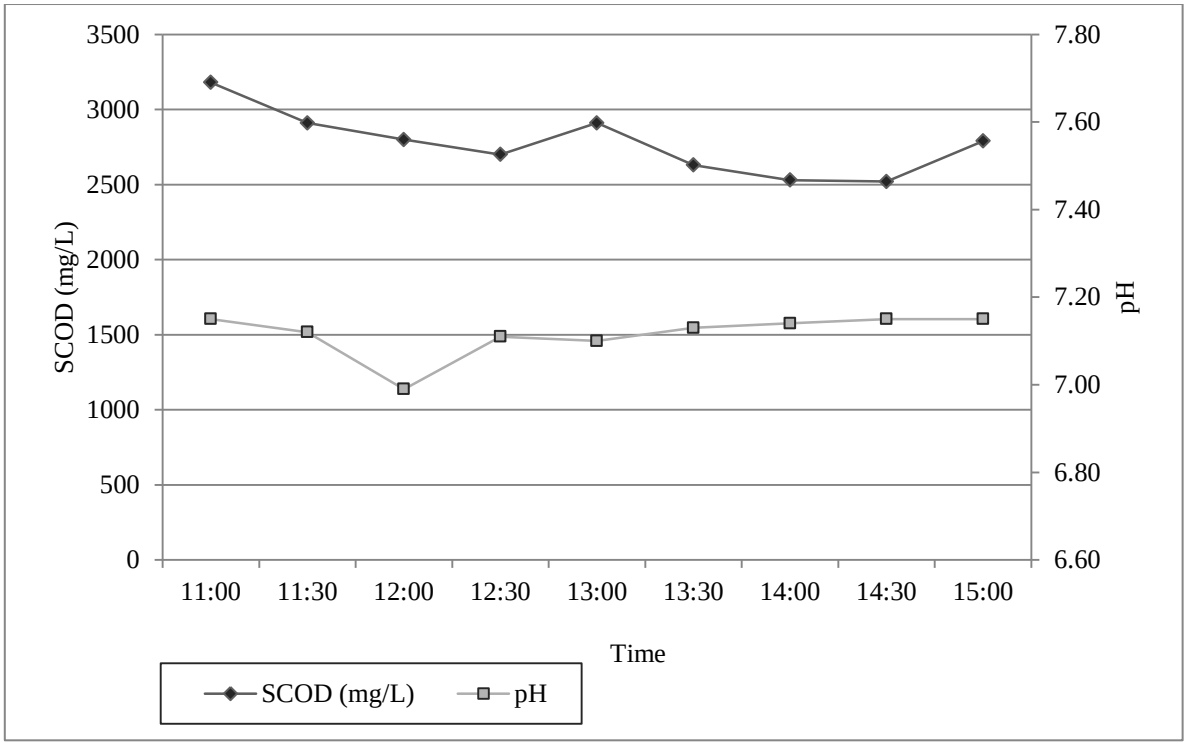

Figure 6.27: SCOD and pH tracking study, 36 g VS/day

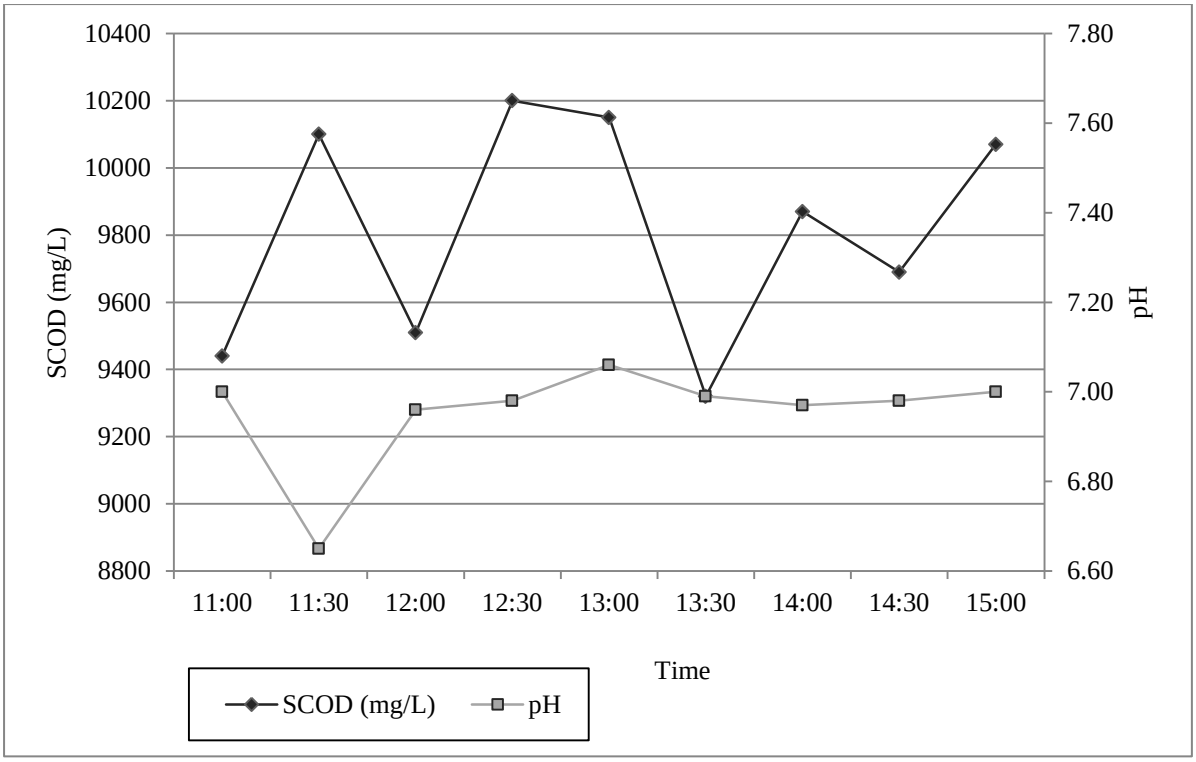

Figure 6.28: SCOD and pH tracking study, 48 g VS/day

7 Conclusions

The results from both Phase I and II demonstrate that coconut copra is amenable to anaerobic digestion and the methane yields achieved for the batch reactor and CSTR systems were comparable to other FVWs and high lipid substrates. The high fat content of the coconut copra makes it potentially a good substrate for anaerobic digestion because of the higher theoretical methane yields achievable in comparison to carbohydrates and proteins. The overall results from the digestion of coconut copra therefore suggest that this substrate does have potential as a sustainable and high energy resource for PICs when digested using anaerobic technologies. However, further research is required to in order to progress towards integration of this substrate in larger scale, reactor models.

7.1.1 Summary of Results: Phase I

- The optimal OLR range for the batch systems was determined to be very limited. The maximum methane production was observed to be 0.420 CH_4/g VS which was achieved at the OLR of 3.6 g VS (2.4 g VS/L Rctr). OLRs higher than 15 g VS (10 g VS/ L Rctr) resulted in reactor souring and unfavourable environmental conditions for methanogenic activity.

- The inhibition of the anaerobic digestion process at OLRs exceeding 15 g VS was possibly the result of toxicity to methanogenic bacteria from LCFA as VFA concentrations were present in the final sludge readings in low concentrations.

7.1.2 Summary of Results: Phase II

- By extending the HRT/SRT to 200 days in the CSTRs, washout caused by lipid molecules adhering to the biomass was successfully prevented. A stable reactor system was eventually achieved through gradual increases to OLRs while an accelerated start-up could not be achieved due to a rapid decline in pH values which were outside the optimal range for methanogenic activity.

- Favourable environmental conditions for anaerobic digestion were able to be maintained in CSTRs up to an OLR of 48 g VS/day (2.4 g VS/ L·day). The relatively neutral pH and high mean methane production rate observed of 0.708 L CH_4/ g VS·day also suggests that the OLR could be potentially increased further than the OLRs tested in Phase II.

- An increase in mixing frequency had a positive effect on methane production and was observed to be an important parameter in maintaining contact of the biomass with the coconut substrate, especially in consideration of its propensity to form floating aggregates.

- Additional supplements of $(NH_4)_2HPO_4$ failed to increase biological activity and improve on the C:N:P ratio of the coconut substrate. Consequently, an accumulation of excess nitrogen resulted in concentrations of NH_3 that were toxic to methanogens and ultimately resulted in reactor failure.

- The CSTRs were able to achieve higher percentage of the theoretical methane production than the batch systems possibly due to longer acclimation periods for anaerobic bacteria which assisted in preventing toxicity from LCFAs.

7.1.3 Summary of Results: VFA and SCOD Tracking Studies

- Low VFA concentrations coupled with high levels of CO_2 partial pressure identified at the higher OLRs in the batch reactors suggests that dissolved $(CO_2)_{aq}$ and its dissociation to carbonic acid could be attributed to the low pH values which were observed to be unfavourable for methanogenic activity.

- The results from the SCOD tracking studies from the CSTRs demonstrated that with an increase in OLR there was an observed increase in SCOD concentrations and lag time in methane production up to 9 hours immediately after feeding at the OLRs of 36 and 48 g VS/day.

- The VFA concentrations in both the batch reactors and CSTRs were tested below levels toxic to methanogenic activity. The increasing SCOD concentrations which coincided with a decline in methane production in failed reactors suggests that other soluble by-products, including LCFAs, may be attributed to the inhibitions observed in anaerobic digestion process for both systems.

8 Further Research and Recommendations

The anaerobic digestion of the coconut copra was successfully achieved in both the batch reactors and CSTRs however the high lipid content of the coconut copra contributed to a number of limitations of the anaerobic digestion process. It is recommended that future research focus on areas which could assist in resolving the fundamental issues associated with inhibition of the microbial population from LCFAs, improved solubilisation of the coconut copra and prevention in the accumulation of suspended FOG layers.

The following recommendations could be beneficial in resolving these issues and improve further on the results achieved during this research.

- The addition of lipase enzymes or pre-treatment methods to increase the hydrolysis of the lipid fraction of the coconut substrate could be integrated into the systems process. This could potentially help solubilise the lipid content and resolve problems associated with floating coconut aggregates therefore assisting in increased contact between the substrate and biomass.

- Increasing the temperature at which the anaerobic reactors are operated at from mesophilic to thermophilic (40-50°C) may also increase the solubilisation and hydrolysis of the coconut substrate. Recent research into the thermodynamics of LCFAs degradation by Oh and Martín-González (2010) has reported that fatty acids may be better digested if facilitated by the provision of supplemental thermal energy (Oh and Martin 2010). In addition, the predominate fatty acid in the coconut copra (lauric acid) has a low melting point of 43.2°C therefore at thermophilic temperatures this component should be present in a liquid form and not as a particulate.

- Investigations into the use of acclimated inoculums could potentially prevent the inhibition of methane production from negative effects of LCFAs on microbial communities. Long acclimation periods may also assist in promoting the growth of syntrophic LCFA-degraders which could be potentially useful for faster reactor start-up as this species of bacteria typically have slow growth rates.

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10 Appendices

A. Elemental composition of coconut copra

Components	Units	Coconut copra (per 100g)	
		Raw coconut	Dried coconut
Moisture content*	%	51.45	2.90
Volatile solids (VS)	%	97.80	97.95
Ash	%	0.90	1.40
COD (total)**	g O_2		37
Nitrogen (total)	g		1.24
Phosphorus (total)	g		0.19
C: N : P ratio			29.9 : 1: 0.14
Energy ***	Calories	354	660
Protein	g	3.5	7.2
Fat (total) (saturated)	g	33.50 29.70	64.53 7.22
Carbohydrates - Fibre		9.4	23.0 3.90
Potassium	mg	356.00	543.00
Sodium	mg	20.00	37.00
Calcium	mg	13.00	26.00

*Values determined from tests laboratory testing using Standard Methods
**External accredited laboratory tests
***Nutritional information obtained from manufacturer

B. Fatty acid composition of coconut copra

Fatty acid	Number of Carbons	Percentage of total lipids (%)
Caprylic	8	4.34
Capric	10	6.22
Lauric	12	48.6
Myristic	14	19.2
Palmitic	16	9.64
Stearic	18	3.23

Source: (Santoso et al. 1996)

C. Raw data for sludge inoculum

Table 10.1: Inoculum pH, alkalinity, TS, VS, TSS and VSS data

Sample number	pH	Alkalinity (mg/L as $CaCO_3$)	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
1	7.92	4600	16224	11296	8760	10618
2	8.14	4500	16486	11416	12040	11660
3	7.46	4520	17020	12012	14680	12060
4	7.80	5130	17844	12544	14550	11820
5	7.40	5000	17456	12392	12780	11798
6	7.22	4520	17968	12544	12180	11245
7	7.32	5140	26496	16500	13900	12650
8	7.61	5260	25760	16536	14250	11470
9	7.32	4860	12904	8812	13890	12400
Mean	7.58	4837	18684	12672	13003	11747
± Stdev	0.30	290	4228	2321	1764	573

D. Raw data for methane/carbon dioxide percentages and yields: Phase I, batch reactors

Table 10.2:Methane percentages and yield data , Phase I, batch reactors: Control and Tests 1-7

Methane %	Controls*	Test 1		Test 2		Test 3		Test 4		Test 5		Test 6		Test 7	
HRT (days)		102 g DF	102 g DF	102 g R	102 g R	80 g DF	80 g DF	60 g DF	60 g DF	40 g DF	40 g DF	25 g DF	25 g DF	15 g DF	15 g DF
1	0	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0
2	3.7	24.8	14.3	16.2	29.0	17.2	19.2	21.5	18.1	16.1	17.5	12.4	12.5	13.0	11.8
3	5.2	22.8	10.0	14.0	25.5	22.4	17.7	20.9	26.6	17.7	19.1	14.3	14.2	17.2	15.2
4	7.1	15.5	8.3	8.1	21.6	20.8	15.5	19.8	23.0	15.6	15.8	14.5	12.1	14.4	16.0
5	12.2	13.4	8.4	16.2	14.5	16.2	14.5	16.5	16.4	10.4	12.0	11.8	8.4	13.6	11.0
6	11.3	12.5	8.3	13.0	14.0	13.0	14.0	14.1	12.1	9.3	10.1	10.5	5.0	11.9	7.9
7	9.7	12.5	7.8	7.8	9.8	7.8	9.8	8.9	5.6	4.3	4.7	10.0	5.0	11.2	7.0
8	8.1	11.0	5.8	4.8	5.8	4.8	5.8	3.9	3.6	3.5	3.6	9.2	4.1	9.4	5.5
Mean (%)	8.2	16.1	9.0	11.4	17.2	14.6	13.8	15.1	15.1	11.0	11.8	11.8	8.8	13.0	10.6
Stdev (%)	3.1	5.5	2.6	4.5	8.5	6.5	4.6	6.6	8.5	5.7	6.1	2.1	4.2	2.5	4.0
Mean biogas yield (L)	0.00	8.17		8.04		6.90		6.20		2.60		1.70		1.85	
Mean methane yield (L/gVS)	0.000	0.013	0.007	0.009	0.014	0.013	0.012	0.012	0.012	0.007	0.008	0.005	0.004	0.014	0.012
Stdev methane yield (L/gVS)	0.000	0.004	0.002	0.004	0.007	0.006	0.004	0.007	0.009	0.004	0.004	0.001	0.003	0.007	0.007
Mean CO_2(%)	10.5	50.0	47.1	23.3	54.1	47.1	59.3	57.9	48.8	50.1	52.3	55.3	48.8	40.7	38.0
Stdev CO_2 (%)	3.0	0.4	0.2	5.6	2.9	18.8	2.4	3.6	8.4	4.4	5.9	5.8	3.6	3.9	8.4

*Refer to Tables 10.4 and 10.5 for calculations of mean values

Table 10.3:Methane percentages and yield data, Phase I, batch reactors: Control and Tests 8-14

HRT (days)	Test 8		Test 9		Test 10		Test 11		Test 12		Test 13		Test 14	
	12 g DF	12 g DF	9 g DF	9 g DF	6 g DF	6 g DF	6 g R	6 g R	6 g DS	6 g DS	3.6 g DF	3.6 g DF	1.8 g DF	1.8 g DF
1	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0
2	11.7	11.0	9.2	11.9	13.3	10.6	15.4	11.4	5.4	5.7	12.5	13.5	4.3	2.4
3	15.8	15.4	16.7	20.1	24.2	20.8	28.2	21.6	8.8	11.5	24.8	27.9	9.5	8.6
4	7.7	8.7	14.9	17.5	35.1	41	38.2	37.9	13.9	10	31.5	37.7	10.1	13.1
5	11.4	11.3	16.4	18.7	57.6	55.1	45.9	48.3	13.3	12.3	42.8	48.5	13	15.2
6	9.7	9.4	15.4	23.6	66.3	64.2	58.2	53.6	13.1	11.4	49.4	53.7	15.9	23.5
7	10.1	12.0	15.0	22.3	67.6	68.5	61.2	63.8	16.9	11	54	56.2	11.5	24.6
8	6.8	5.3	14.0	22.0	69.8	71.8	67.4	68.1	9.9	15.7	60.4	58.8	20.3	21.3
Mean (%)	10.5	10.4	14.5	19.4	47.7	47.4	44.9	43.5	11.6	11.1	39.3	42.3	12.1	15.5
Stdev (%)	3.0	3.1	2.5	4.0	23.2	24.1	18.9	21.1	3.8	3.0	17.2	16.8	5.1	8.2
Mean biogas yield (L)		1.80		1.50		3.50		2.55		1.10		3.70		1.10
Mean methane yield (L/gVS)	0.016	0.016	0.021	0.028	0.278	0.277	0.191	0.185	0.021	0.020	0.404	0.435	0.074	0.095
Stdev methane yield (L/gVS)	0.004	0.005	0.004	0.007	0.135	0.141	0.080	0.090	0.007	0.005	0.176	0.173	0.002	0.004
Mean CO_2(%)	36.1	35.6	37.1	32.0	12.0	26.5	8.1	20.9	10.1	18.3	25.4	26.5	18.4	20.4
Stdev CO_2 (%)	14.9	3.4	7.8	8.0	4.4	6.8	4.0	9.7	5.5	9.0	5.8	5.3	7.6	9.0

Table 10.4: Control sample methane percentages data (Controls from Tests 1-8 used)

Controls								
HRT (days)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	2.9	4.1	5.3	3.4	5.3	2.1	3.0	3.7
3	2.7	5.3	7.0	5.2	7.0	3.2	6.2	5.2
4	2.9	6.0	10.5	6.3	10.5	4.2	9.0	7.1
5	16.5	16.4	13.4	8.4	13.4	4.7	12.3	12.2
6	14.1	12.1	12.5	8.3	12.5	5.0	14.7	11.3
7	8.9	5.6	12.5	7.8	12.5	5.2	15.3	9.7
8	3.9	3.6	11.0	5.8	11.0	4.9	16.4	8.1
Mean methane (%)	7.4	7.6	10.3	6.5	10.3	4.2	11.0	8.2
Stdev methane (%)	5.8	4.8	3.0	1.8	3.0	1.1	5.1	3.5

Table 10.5: Control sample carbon dioxide percentages data (Controls from Tests 1-8 used)

Controls								
HRT (days)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	10.5	6.9	7.7	6.8	7.7	7.0	6.0	7.5
3	3.0	7.0	9.1	8.4	9.1	11.1	9.6	8.2
4	3.6	7.3	12.3	9.2	12.3	10.0	12.0	9.5
5	3.8	9.0	14.5	10.8	14.5	10.2	13.9	11.0
6	4.2	9.4	16.4	11.8	14.4	10.4	15.3	11.7
7	4.2	13.2	17.4	12.8	16.4	10.4	15.8	12.9
8	3.9	14.3	11.0	14.8	17.4	10.4	16.2	12.6
Mean CO_2 (%)	4.7	9.6	12.6	10.7	13.1	9.9	12.7	10.5
Stdev CO_2(%)	2.6	3.0	3.7	2.7	3.6	1.3	3.8	3.0

Table 10.6: Mean methane percentages and yield data, Phase I, batch reactors: Control and Tests 1-14

HRT (days)	Test number													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	19.6	22.6	18.2	19.8	16.8	12.5	12.4	11.4	10.6	12.0	13.4	5.6	13.0	3.4
3	16.4	19.8	20.1	23.8	18.4	14.3	16.2	15.6	18.4	22.5	24.9	10.2	26.4	9.1
4	11.9	14.9	18.2	21.4	15.7	13.3	15.2	8.2	16.2	38.1	38.1	12.0	34.6	11.6
5	10.9	15.4	15.4	16.5	11.2	10.1	12.3	11.4	17.6	56.4	47.1	12.8	45.7	14.1
6	10.4	13.5	13.5	13.1	9.7	7.8	9.9	9.6	19.5	65.3	55.9	12.3	51.6	19.7
7	10.2	8.8	8.8	7.3	4.5	7.5	9.1	11.1	18.7	68.1	62.5	14.0	55.1	18.1
8	8.4	5.3	5.3	3.8	3.6	6.7	7.5	6.1	18.0	70.8	67.8	12.8	59.6	20.8
Mean	12.5	14.3	14.2	15.1	11.4	10.3	11.8	10.5	17.0	47.6	44.2	11.4	40.8	13.8
Stdev	4.1	6.5	5.6	7.6	5.9	3.1	3.3	3.0	3.2	23.6	20.0	3.4	17.0	6.6
Mean biogas yield	8.17	8.04	6.90	6.20	2.60	1.70	1.85	1.80	1.50	3.5	2.55	1.10	3.70	1.10
Mean methane yield (L/gVS)	0.010	0.011	0.012	0.012	0.007	0.004	0.013	0.016	0.025	0.277	0.188	0.021	0.420	0.084
Stdev methane yield (L/gVS)	0.003	0.005	0.005	0.008	0.004	0.002	0.007	0.005	0.005	0.138	0.085	0.006	0.174	0.003
Mean CO_2(%)	48.5	38.7	53.2	53.3	51.2	52.0	39.3	35.8	34.5	19.3	14.5	14.2	25.9	19.4
Stdev CO_2 (%)	0.3	4.3	10.6	6.0	5.2	4.7	6.2	9.2	7.9	5.6	6.9	7.2	5.5	8.3
Ratio CH_4: CO_2	3.871	2.701	3.748	3.539	4.491	5.060	3.334	3.428	2.035	0.405	0.328	1.252	0.635	1.402

Table 10.7: Measured concluding values of batch reactors data, SCOD,TS, VS, VSS and pH (DF coconut)

Test number											
Concluding values	**1**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**13**	**14**
SCOD (mg/L)	15320	12600	11750	10500	10090	10150	10585	10500	5075	1275	1700
TS (mg/L)	96688	54252	52460	38380	33426	28472	17544	11532	18508	21664	6032
VS (mg/L)	51936	36056	35720	34336	26574	16084	12496	9636	11272	12544	4116
VSS (mg/L)	34370	19740	25933	29810	34370	18910	9000	6810	7060	10110	2850
Alkalinity (mg/L as $CaCO_3$)	1520	2395	2770	2590	2587	3150	3840	4285	4270	5235	4505
pH	5.35	5.25	6.05	6.20	6.40	6.95	6.95	6.52	7.12	7.31	7.65
Ratio VS/TS	0.537	0.665	0.681	0.895	0.795	0.678	0.712	0.662	0.580	0.579	0.687
VSS/VS ratio	0.662	0.547	0.726	0.868	0.795	0.892	0.720	0.892	0.626	0.806	0.692
VS destruction (%)	45.3	38.9	49.2	65.2	70.5	58.1	50.6	44.5	60.4	77.1	28.4

E. Raw data for methane percentages and yields: Phase II CSTRs

Table 10.8: Gas production and percentages, pH and alkalinity; CSTRs Run 1

Day	CH_4 (%)		CO_2 (%)		O_2 (%)		Balance (%)		pH		Alkalinity (mg/L $CaCO_3$)	
	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2
1	6.6	9.2	8.2	9.2	0.2	0	85	81.6	7.44	7.45	4320	4660
2	20.9	25.9	18.7	20.0	0.3	0.3	59.9	54.1	7.44	7.43	4260	4240
3	39.2	42.8	24.6	20.0	0.3	0	35.8	31.8	7.45	7.37	4220	4420
4	46.9	51.2	28.8	29.1	0.3	0	24.3	19.6	7.35	7.31	4060	4060
5	52.3	55.4	32.2	31.9	0.4	0	16.4	12.7	7.30	7.32	3820	3760
6	56.4	58.5	34.5	34.2	0.3	0	10.9	7.7	7.23	7.21	3380	3400
7	58.0	59.5	36.5	35.6	0.2	0.1	8.8	5.8	7.16	7.18	3100	3160
8	63.0	66.0	35.5	37.3	0	0.1	5.5	3.4	7.19	7.18	3460	3680
9	64.0	65.0	33.0	33.1	0.1	0	2.4	0.8	7.25	7.33	3700	3780

Table 10.9: Gas production and percentage, pH and alkalinity; CSTRs Run 2

Day	CH_4 (%)		CO_2 (%)		O_2 (%)		Balance (%)		pH		Alkalinity (mg/L $CaCO_3$)		OLR (gVS)		Gas yield (L)		Methane production (LCH_4/day)	
	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2
1	6.7	3.2	13.2	11.6	0	0	80	85.2	7.54	7.67	5060	4920	12	12	1.7	1.9	0.0095	0.0051
2	33.5	12.8	20.6	15.7	0.2	0	45.6	71.5	7.48	7.64	4800	4800	12	12	4.8	1.3	0.1340	0.0139
3	53.8	35.9	27.6	25.0	0.1	0	18.3	38..1	7.38	7.45	4600	4680	12	12	4.3	0.5	0.1928	0.0150
4	60.6	47.5	32.3	31.2	0	0	7.8	21.3	7.26	7.38	4600	4480	12	12	4.5	5.4	0.2273	0.2138
5	63.3	55.0	34.2	36.0	0	0.1	1.5	8.8	7.42	7.24	4680	4520	12	12	6.7	7.7	0.3534	0.3529
6	66.3	59.6	32.7	38.2	0	0	1.2	2	7.39	7.29	4680	4300	12	12	8.1	9.6	0.4475	0.4768
7	65.0	60.0	34.6	40.0	0	0	0.6	0.8	7.38	7.11	4480	4080	12	12	12.3	10.8	0.6663	0.5400
8	68.1	59.4	32.9	40.1	0	0	0	0.5	7.41	7.09	4520	4000	12	12	13.8	15.2	0.7832	0.7524
9	70.1	63.6	31.1	36.7	0	0	0	0	7.45	7.09	4580	3760	12	12	13.7	14.1	0.8003	0.7473
10	71.5	67.2	30.1	34.2	0.2	0	0	0	7.44	7.25	4560	3500	12	12	13.4	15.4	0.7984	0.8624
11	70.5	70.6	30.4	29.6	0.2	0	0	0	7.42	7.27	4560	3470	12	12	12.4	16.6	0.7285	0.9766
12	70.3	74.3	30.8	26.7	0.2	0	0	0	7.45	7.37	4460	3820	12	12	14.8	20.2	0.8670	1.2476
13	70.6	76.7	30.3	25.0	0.2	0	0	0	7.40	7.52	4620	4200	12	12	13.6	20.2	0.8001	1.2879
14	69.9	76.9	31.5	25.0	0.1	0	0	0	7.37	7.46	4420	4560	12	12	12.9	17.9	0.7514	1.1439
15	69.9	72.5	31.5	28.1	0.2	0	0	0	7.53	7.44	4460	4560	12	12	13.2	17.9	0.7689	1.0784
16	69.8	70.0	31.7	30.0	0.2	0	0	0	7.33	7.35	4500	4500	12	12	12.1	15.1	0.7038	0.8909
17	69.0	70.6	32.2	29.4	0.2	0	0	0	7.40	7.40	4540	4540	12	12	12.1	13.9	0.6958	0.8178
18	69.0	70.0	31.7	31.6	0.1	0	0	0	7.40	7.41	4460	4510	12	12	14.5	13.1	0.8338	0.7642
19	69.0	70.0	31.9	30.0	0.1	0	0	0	7.27	7.44	4500	4500	12	12	14.5	15.3	0.8338	0.8836
20	68.8	69.3	32.0	31.2	0.1	0	0	0	7.24	7.41	4480	4540	12	12	14.1	15.3	0.8084	0.8836
21	69.0	68.4	31.7	32.1	0	0	0	0	7.47	7.44	4580	4500	12	12	13.8	15.3	0.7935	0.8721

Day	CH_4 (%)		CO_2 (%)		O_2 (%)		Balance (%)		pH		Alkalinity (mg/L $CaCO_3$)		OLR (gVS)		Gas yield (L)		Methane production (LCH_4/day)	
22	68.2	69.3	32.1	31.5	0.1	0	0	0	7.31	7.44	4480	4480	12	12	12.9	13.4	0.7332	0.7739
23	68.6	68.8	32.4	31.6	0.2	0	0	0	7.33	7.42	4520	4300	12	12	14.3	16.7	0.8175	0.9575
24	68.5	69.4	32.0	31.3	0.2	0	0	0	7.45	7.37	4460	4520	12	12	14.3	13.9	0.8163	0.8039
25	69.0	69.0	31.4	32.3	0.3	0	0	0	7.38	7.42	4340	4280	24	12	17.5	12.5	0.5031	0.7188
26	68.2	69.0	32.9	32.3	0.2	0	0	0	7.37	7.55	4240	4460	24	12	20.7	14.2	0.5882	0.8165
27	68.6	67.1	31.8	32.7	0.2	0	0	0	7.37	7.61	4160	4560	24	12	25.1	15.9	0.7174	0.8891
28	68.9	67.6	32.6	32.6	0.1	0	0	0	7.45	7.49	4200	4480	24	12	24.0	14.8	0.6890	0.8337
29	68.7	69.0	32.6	32.0	0.1	0	0	0	7.53	7.33	4180	4480	24	12	24.0	14.0	0.7401	0.8050
30	66.0	68.8	34.4	32.0	0.2	0	0	0	7.52	7.41	4450	4380	24	24	24.0	14.2	0.7401	0.4082
31	69.2	67.6	32.2	33.1	0.2	0	0	0	7.50	7.39	4285	4280	24	24	23.9	17.4	0.7401	0.4901
32	69.3	70.2	32.0	29.8	0.1	0	0	0	7.31	7.55	4120	3750	24	24	27.4	29.1	0.7912	0.8497
33	70.5	70.0	31.5	30.0	0.2	0	0	0	7.50	7.38	3990	3960	24	24	23.0	29.1	0.6745	0.8473
34	69.5	68.7	31.5	31.9	0.1	0	0	0	7.40	7.40	3860	3980	24	24	21.6	23.6	0.6255	0.6756
35	69.8	69.0	30.9	32.2	0.1	0	0	0	7.47	7.43	3900	3880	24	24	31.6	26.4	0.9176	0.7590
36	69.8	67.9	32.0	32.4	0.2	0	0	0	7.41	7.42	3940	3960	24	24	31.6	25.2	0.9176	0.7130
37	68.2	68.8	32.2	31.5	0	0	0	0	7.39	7.45	3920	3480	24	24	22.7	24.5	0.6451	0.7023
38	69.0	69.0	31.6	31.0	0.2	0	0	0	7.42	7.36	3300	4200	24	24	28.2	26.3	0.8108	0.7561
39	68.6	69.0	32.2	32.0	0.2	0	0	0	7.41	7.38	3920	3940	24	24	25.7	25.3	0.7346	0.7274
40	68.5	69.0	31.9	32.0	0.2	0	0	0	7.44	7.40	3840	3900	24	24	27.2	25.9	0.7763	0.7446
41	68.6	68.2	32.4	31.6	0	0	0	0	7.39	7.37	3880	3860	24	24	25.2	23.7	0.7203	0.6735
42	68.4	68.3	32.2	32.0	0.1	0	0	0	7.36	7.32	3840	3960	24	24	26.8	27.1	0.7638	0.7718
43	69.2	69.8	32.1	32.0	0.1	0	0	0	7.38	7.39	3920	3940	24	24	25.6	27.0	0.7381	0.7853
44	68.8	68.8	32.9	31.9	0.2	0	0	0	7.39	7.40	3900	3600	24	24	23.9	23.0	0.6851	0.6593
45	68.1	68.1	32.6	31.7	0.2	0	0	0	7.30	7.42	3700	3660	24	24	24.8	27.6	0.7037	0.7832
46	69.3	68.1	32.0	31.6	0.1	0.1	0	0	7.40	7.44	3640	3720	24	24	26.0	27.4	0.7508	0.7786
47	69.0	68.6	32.7	32.1	0.1	0	0	0	7.39	7.25	3680	3800	24	24	24.1	24.3	0.6929	0.6946
48	68.2	68.6	32.4	32.2	0.2	0	0	0	7.40	7.27	3765	3760	24	24	24.1	25.1	0.6848	0.7174

Day	CH_4 (%)		CO_2 (%)		O_2 (%)		Balance (%)		pH		Alkalinity (mg/L $CaCO_3$)		OLR (gVS)		Gas yield (L)		Methane production (LCH_4/day)	
49	68.8	69.2	32.3	31.5	0.1	0	0	0	7.41	7.24	3850	3640	24	24	27.4	29.7	0.7855	0.8564
50	69.2	68.4	32.3	33.6	0.1	0	0	0	7.21	7.33	3640	3720	24	24	26.4	25.4	0.7612	0.7239
51	68.0	67.0	32.1	33.0	0.1	0	0	0	7.22	7.24	3700	3600	36	24	21.3	21.1	0.4023	0.5890
52	67.8	67.4	32.6	32.4	0	0	0	0	7.19	7.30	3500	3640	36	24	30.6	31.5	0.5763	0.8846
53	69.5	65.5	31.5	30.1	0.1	0	0	0.3	7.23	7.22	3500	3600	36	24	40.6	31.0	0.7838	0.8460
54	68.3	64.0	33.4	3.3	0.1	0	0	0.2	7.30	7.20	3380	3590	36	24	35.0	31.7	0.6640	0.8453
55	66.2	68.1	34.1	31.8	0.1	0	0	0	7.21	7.28	3280	3600	36	24	33.3	34.9	0.6124	0.9903
56	68.6	72.9	32.4	28.1	0	0.1	0	0	7.27	7.42	3300	3580	36	24	31.1	36.1	0.5917	1.0965
57	66.1	69.5	34.9	30.9	0	0.1	0	0	7.38	7.36	3400	3340	36	24	31.7	32.1	0.5820	0.9296
58	72.9	68.5	27.9	31.5	0	0	0	0	7.42	7.35	3200	3560	36	36	35.4	40.3	0.7169	0.7668
59	71.6	69.6	29.4	30.8	0	0	0	0	7.38	7.38	3560	3660	36	36	27.7	43.9	0.5509	0.8487
60	68.9	70.0	31.9	30.6	0.2	0	0	0	7.42	7.35	3360	3600	36	36	37.1	37.4	0.7101	0.7272
61	71.9	69.6	29.2	31.0	0	0	0	0	7.39	7.33	3540	3540	36	36	40.3	44.3	0.8049	0.8565
62	71.1	70.0	30.6	30.0	0	0	0	0	7.41	7.36	3100	3540	36	36	34.4	43.9	0.6794	0.8536
63	68.3	70.0	32.4	30.0	0	0	0	0	7.35	7.34	3400	3600	36	36	31.2	40.3	0.5919	0.7836
64	69.0	70.2	30.9	29.6	0	0	0	0	7.36	7.38	3435	3780	36	36	32.6	42.3	0.6248	0.8249
65	70.1	69.0	30.0	31.8	0	0	0	0	7.42	7.39	3300	3600	36	36	39.3	41.9	0.7653	0.8021
66	68.6	68.8	31.3	32.0	0	0	0	0	7.40	7.36	3240	3580	36	36	25.4	41.4	0.4840	0.7912
67	69.3	69.4	31.1	31.1	0.1	0	0	0	7.36	7.32	3200	3600	36	36	32.6	44.5	0.6276	0.8579
68	69.0	70.0	31.0	30.0	0.1	0	0	0	7.31	7.31	3300	3400	36	36	30.3	46.1	0.5808	0.8964
69	68.3	69.5	32.1	31.1	0	0	0	0	7.27	7.34	3220	3520	36	36	32.2	44.5	0.6100	0.8591
70	70.4	68.7	31.7	32.2	0.1	0	0	0	7.36	7.28	3180	3360	36	36	34.0	40.9	0.6649	0.7805
71	70.5	71.4	31.5	30.5	0.2	0	0	0	7.32	7.25	3090	3500	36	36	37.9	39.2	0.7422	0.7775
72	68.8	68.8	32.0	31.9	0.1	0	0	0	7.29	7.31	3000	3440	36	36	30.7	31.3	0.5867	0.7260
73	69.3	70.6	31.3	30.2	0.1	0	0	0	7.35	7.30	3400	3400	36	36	30.2	34.4	0.5814	0.6746
74	69.3	68.7	32.4	31.6	0	0	0	0	7.24	7.35	3100	3420	36	36	33.5	37.9	0.6449	0.7233
75	70.3	68.8	31.5	30.8	0	0	0	0	7.25	7.20	3020	3120	36	36	32.1	38.3	0.6268	0.7320

Day	CH_4 (%)		CO_2 (%)		O_2 (%)		Balance (%)		pH		Alkalinity (mg/L $CaCO_3$)		OLR (gVS)		Gas yield (L)		Methane production (LCH_4/day)	
76	69.2	68.4	31.9	32.2	0.1	0	0	0	7.22	7.16	3000	3170	36	36	33.8	34.6	0.6497	0.6574
77	69.0	67.7	31.9	32.1	0	0	0	0	7.20	7.18	3270	3200	48	36	31.1	34.2	0.4471	0.6432
78	69.0	68.8	31.0	31.6	0	0	0	0	7.23	7.16	3380	3140	48	36	41.0	33.4	0.5894	0.6383
79	69.0	68.6	31.6	31.6	0	0	0	0	7.29	7.11	3160	3000	48	36	50.0	28.7	0.7188	0.5469
80	69.0	68.4	31.0	32.6	0	0	0	0	7.29	7.15	3220	2940	48	36	51.0	43.0	0.7331	0.8170
81	69.0	69.0	32.0	31.6	0	0	0	0	7.32	7.15	3210	2940	48	36	51.0	38.9	0.7331	0.7456
82	69.0	68.9	33.6	32.0	0	0	0	0	7.35	7.15	3260	2900	48	36	51.0	34.9	0.7331	0.6679
83	70.8	69.2	30.4	32.6	0.1	0	0	0	7.35	7.11	3280	2840	48	48	50.0	35.0	0.7375	0.5039
84	69.5	68.6	32.0	31.7	0.1	0	0	0	7.35	7.19	3240	2820	48	48	59.0	35.0	0.8543	0.5002
85	63.1	69.3	3.0	31.2	0.1	0	0	0	7.41	7.16	3180	2800	48	48	56.6	36.5	0.7441	0.5185
86	64.0	67.8	35.8	31.9	0.2	0	0	0.3	7.25	7.11	3420	2600	48	48	18.0	38.0	0.2400	0.5368
87		68.2		31.9		0		0.8		7.05		2600		48		46.8		0.6650
88		67.7		31.0		0		0.6		7.05		2580		48		44.2		0.6234
89		68.7		32.0		0		0		7.03		2400		48		44.7		0.6398
90		69.5		31.6		0		0		7.00		2380		48		50.0		0.7240
91		68.0		31.6		0.1		0		7.07		2580		48		57.5		0.8146
92		70.1		31.4		0		0		7.04		2600		48		50.7		0.7404
93		68.5		31.3		0		0		7.03		2600		48		55.9		0.7977
94		68.9		31.3		0		0		7.00		2580		48		55.0		0.7895
95		69.3		31.7		0		0		7.02		2580		48		52.8		0.7623
96		68.6		31.5		0		0		7.01		2640		48		53.1		0.7589
97		69.3		32.9		0		0		7.06		2640		48		46.1		0.6656
98		69.0		31.6		0		0		7.08		2200		48		48.2		0.6929
99		69.0		31.6		0		0		7.07		2500		48		52.0		0.7475
100		69.0		31.6		0		0		7.09		2600		48		50.6		0.7274
101		68.1		33.2		0		0		7.12		2640		48		50.6		0.7475
102		70.7		30.3		0		0		7.11		2640		48		50.6		0.7179

Day	CH_4 (%)		CO_2 (%)		O_2 (%)		Balance (%)		pH		Alkalinity (mg/L $CaCO_3$)		OLR (gVS)		Gas yield (L)		Methane production (LCH_4/day)	
103		70.6		30.0		0		0		7.10		2640		48		58.6		0.8631
104		64.8		32.6		0		0		7.12		2700		48		53.8		0.7913

Table 10.10: Gas production and percentage, pH and alkalinity; CSTRs Run 3

Day	CH_4 (%)	CO_2 (%)	O_2 (%)	Balance (%)	pH	Alkalinity (mg/L $CaCO_3$)	OLR (gVS)	Gas yield (L)	Methane production (LCH_4/day)
1	38.0	24.2	0.2	36.8	7.52	4500	12	6.2	0.1963
2	48.6	29.9	0.1	21.5	7.39	4920	12	4.6	0.1863
3	55.3	35.1	0.1	9.4	7.38	4860	12	6.0	0.2765
4	56.2	41.1	0.1	2.5	7.28	4600	24	9.1	0.2131
5	53.0	44.6	0.2	2.1	7.18	4400	24	12.9	0.2849
6	53.0	45.4	0.3	1.6	7.11	4440	24	16.7	0.3688
7	53.7	45.5	0.0	0.9	7.01	3800	24	12.3	0.2752
8	51.3	48.4	0.3	0.3	6.80	3850	24	8.9	0.2232
9	52.0	46.2	0.1	1.5	6.94	3900	24	7.9	0.1712
10	49.2	47.0	0.0	3.2	6.60	4100	24	8.1	0.1969
11	50.4	47.3	0.2	2.0	6.86	4320	12	5.3	0.2226
12	51.0	48.0	0.1	0.0	6.55	4270	12	4.7	0.1903
13	53.4	45.0	0.1	1.6	6.50	4220	12	3.6	0.1679
14	54.2	44.0	0.1	1.0	6.50	4390	12	3.5	0.1581
15	56.0	43.0	0.1	0.7	6.83	4960	12	4.8	0.2240
16	53.0	41.0	0.0	6.0	6.70	4850	12	2.5	0.1103
17	52.0	37.0	0.0	11.0	6.30	4750	12	2.5	0.1103

Table 10.11: Gas production and percentage, pH and alkalinity; CSTRs Run 4

HRT (days)	CH_4 (%)		CO_2 (%)		O_2 (%)		Balance (%)		pH		Alkalinity (mg/L $CaCO_3$)		OLR (gVS)		Gas yield (L)		Methane production (LCH_4/day)	
	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2	Rctr 1	Rctr 2
1	6.6	9.2	8.2	9.2	0.2	0.0	85.0	81.6	7.44	7.45	4320	4660	12	12	10.1	9.8	0.0556	0.0751
2	20.9	25.9	18.7	20.0	0.3	0.3	59.9	54.1	7.44	7.43	4260	4240	12	12	8.0	7.8	0.1393	0.6500
3	39.2	42.8	24.6	20.0	0.3	0.0	35.8	31.8	7.45	7.37	4220	4420	12	12	7.1	6.5	0.2319	0.5417
4	46.9	51.2	28.8	29.1	0.3	0.0	24.3	19.6	7.35	7.31	4060	4060	12	12	7.8	6.9	0.3049	0.5750
5	62.9	64.7	63.2	35.2	0.1	0.0	0.6	0.2	7.14	7.45	3500	3660	12	12	9.5	7.2	0.4980	0.6000
6	63.4	64.7	34.8	34.9	0.1	0.0	1.7	0.4	7.53	7.55	3480	3680	12	12	6.1	7.9	0.3223	0.6583
7	64.0	64.9	34.8	35.0	0.1	0.0	0.9	0.0	7.24	7.20	3460	3820	12	24	6.3	7.9	0.3360	0.3292
8	62.9	62.9	36.4	36.2	0.3	0.1	0.7	1.2	7.14	7.24	3520	3680	24	24	7.5	8.9	0.1966	0.3708
9	60.9	63.0	37.0	36.2	0.1	0.0	1.7	1.5	7.21	7.23	3480	3620	24	24	12.5	11.7	0.3172	0.4875
10	64.3	63.0	35.0	35.5	0.1	0.0	1.2	1.2	7.17	7.28	3460	3590	24	24	16.3	16.3	0.4367	0.6792
11	63.4	65.2	35.5	35.5	0.0	0.0	1.2	0.0	7.18	7.25	3420	3640	24	24	12.7	15.9	0.3355	0.6625
12	64.6	62.2	35.0	35.0	0.3	0.0	0.0	0.0	7.19	7.29	3600	3690	24	24	16.1	17.2	0.4473	0.7146
13	67.3	68.4	32.2	34.8	0.2	0.0	0.4	0.0	7.41	7.36	3500	3620	24	24	19.4	18.4	0.5440	0.7667
14	69.8	68.1	34.9	36.6	0.2	0.0	0.0	0.0	7.32	7.43	3380	3540	24	24	16.9	16.4	0.4915	0.6833
15	65.0	64.7	35.7	35.2	0.2	0.1	0.0	0.0	7.16	7.23	3400	3600	24	24	10.7	10.7	0.2898	0.4458
16	66.2	66.0	33.5	34.3	0.0	0.0	0.0	0.0	7.30	7.28	3520	3650	24	24	19.3	16.9	0.5324	0.7042
17	67.7	66.4	34.0	34.3	0.2	0.0	0.0	0.0	7.23	7.28	3580	3680	24	24	18.1	17.0	0.5106	0.7083
18	59.9	65.0	37.3	34.1	0.3	0.0	2.4	0.7	7.03	7.28	3900	3700	24	24	1.9	26.0	0.0474	1.0833
19	56.7	68.2	38.3	31.9	0.0	0.1	4.5	0.0	6.90	7.25	3980	3800	24	24	0.0	14.7	0.0000	0.6125
20	56.2	65.3	38.3	34.0	0.0	0.2	4.5	0.9	6.90	7.25	3950	3680	24	24	0.0	13.4	0.0000	0.5583
21	55.3	62.7	40.5	36.3	0.0	0.2	4.5	0.7	6.94	7.42	3970	3380	24	24	0.0	17.1	0.0000	0.7125
22	51.7	64.0	43.4	36.6	0.0	0.0	4.9	0.0	6.92	7.23	3920	3310	24	24	0.7	19.5	0.0151	0.8125
23	48.8	64.8	46.3	37.0	0.2	0.0	4.6	0.0	6.97	6.94	3890	2960	24	24	1.0	14.6	0.0000	0.6083
24	47.9	64.9	47.5	35.8	0.1	0.0	4.5	0.0	7.01	6.86	3900	2940	24	24	0.2	16.3	0.0040	0.6792

HRT (days)	CH_4 (%)		CO_2 (%)		O_2 (%)		Balance (%)		pH		Alkalinity (mg/L $CaCO_3$)		OLR (gVS)		Gas yield (L)		Methane production (LCH_4/day)	
25	45.7	63.6	48.5	36.8	0.3	0.0	5.4	0.0	6.93	6.72	3910	2800	24	24	0.5	10.3	0.0095	0.4292
26	43.0	60.0	45.5	40.0	0.3	0.0	6.5	0.2	6.90	6.74	3940	3420	24	24	0.6	10.8	0.0108	0.4500
27		57.0		41.9		0.3		1.4		6.60		3360		24		5.5		0.2292
28		46.4		48.8		0.3		4.4		6.77		5340		12		1.9		0.1583
29		43.5		49.5		0.1		6.8		6.80		5000		12		0.6		0.0500
30		40.5		51.4		0.0		7.6		6.90		4900		12		0.8		0.0667
31		39.7		52.0		0.2		8.6		6.42		4820		12		0.1		0.0083

F. Calculation of theoretical methane production (TMP) of coconut copra

Bushwell's equation :

$$C_nH_aO_bN_c + \left[n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right]H_2O \rightarrow \left[\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right]CH_4 + \left[\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right]CO_2 + cNH_3$$

The fat, carbohydrate and protein components present in 1 g of dried coconut have been separated and the corresponding TMP of each determined in order to calculate the total TMP at each OLR.

Fats :

In 1g dried coconut copra there is approximately 0.65g Fat (represented by Lauirc Acid $C_{12}H_{24}O_2$)

$$C_{12}H_{24}O_2 + \left(12 - \frac{24}{4} - \frac{2}{2}\right)H_2O \rightarrow \left(\frac{12}{2} + \frac{24}{8} - \frac{2}{4}\right)CH_4 + \left(\frac{12}{2} - \frac{24}{8} + \frac{2}{4}\right)CO_2$$

$C_{12}H_{24}O_2$ + 5 H_2O → ≈ 8.5CH_4 + 3.5CO_2

200.32 g $C_{12}H_{24}O_2$ → 136 g CH_4

0.65 g $C_{12}H_{24}O_2$ → 0.441g CH_4

Carbohydrates:

In 1g dried coconut copra there is approximately 0.23g Carbohydrates (represented by glucose $C_6H_{12}O_6$)

$$C_6H_{12}O_6 + \left(6 - \frac{12}{4} - \frac{6}{2}\right)H_2O \rightarrow \left(\frac{6}{2} + \frac{12}{8} - \frac{6}{4}\right)CH_4 + \left(\frac{6}{2} - \frac{12}{8} + \frac{6}{4}\right)CO_2$$

$C_6H_{12}O_6$ → 3 CH_4 + 3 CO_2

180.61 g $C_6H_{12}O_6$ → 48g CH_4

0.23g $C_6H_{12}O_6$ → 0.0611 g CH_4

Protiens :

In 1g dried coconut copra there is approximately 0.07g Protein (represented by $C_4H_{6.1}O_{1.2}N$)

$$C_4\,H_{6.1}O_{1.2}N\ +\left(4-\frac{6.1}{4}-\frac{1.2}{2}+\frac{3\,x1}{4}\right)\,H_2O\ \rightarrow\ \left(\frac{4}{2}+\frac{6.1}{8}-\frac{1.2}{4}-\frac{3\,x1}{8}\ \right)CH_4+\left(\frac{4}{2}-\frac{6.1}{8}+\frac{1.2}{4}+\frac{3\,x1}{8}\right)CO_2+NH_3$$

$C_4H_{6.1}O_{1.2}N$ + 2.5 H_2O	→	≈ 2 CH_4 + 2 CO_2 + NH_3
87.3g $C_4H_{6.1}O_{1.2}N$	→	32g CH_4
0.07g $C_4H_{6.1}O_{1.2}N$	→	0.026g CH_4

Total

1 g of dried coconut copra	→	0.528 g CH_4
1 g VS (dried coconut copra at 98% VS)	→	**0.517 g CH_4**

Conversion of grams of methane at STP to litres at 35°C:

1 mol CH_4	≈	22.4 L CH_4 (STP)
1g CH_4 ≈ 22.4 L/16g mol^{-1}	=	1.4 L CH_4 (STP)
At 35°C CH_4 = 1.4L * (308/273° K)	=	1.579 L CH_4 (35°C)
1 g VS (dried coconut copra)	→	**0.816 L CH_4**

Efficiency (%) = (Actual methane yield at 35 °C/ TMP) x 100

Table 10.12: TMP Phase I, batch reactors

Test	OLR (gVS)	Fats (g)	Carbohydrate (g)	Protein (g)	TMP (L/CH_4)	Average methane production (L/CH_4)	Efficiency (%)
1	102	67.32	23.46	7.34	85.03	1.02	1.2
2	102	67.32	23.46	7.34	85.03	1.15	1.4
3	80	52.80	18.40	5.76	66.70	0.98	1.5
4	60	39.60	13.80	4.32	50.02	0.11	0.2
5	40	26.4	9.20	2.80	33.35	0.29	0.9
6	25	16.50	5.75	1.80	20.84	0.80	3.8
7	15	9.90	3.45	1.08	12.51	0.19	1.5
8	12	7.80	2.76	0.84	10.00	0.16	1.6
9	9	5.85	2.07	0.63	7.50	0.22	3.0
10	6	3.96	1.38	0.43	5.00	1.76	35.2
11	6	3.96	1.38	0.43	5.00	1.10	22.0
12	6	3.96	1.38	0.43	5.00	0.12	2.4
13	3.6	2.34	0.83	0.25	3.00	1.60	53.3
14	1.8	1.17	0.41	0.13	1.50	0.16	10.7

Table 10.13: TMP Phase II, CSTRs at various OLRs, Run 2

OLR (gVS)	Fats (g)	Carbohydrate (g)	Protein (g)	TMP ($L/CH_4 \cdot day$)	Average methane production ($L/CH_4 \cdot day$)	Efficiency (%)
12	7.92	2.76	0.87	8.30	8.30	82.97
24	15.84	5.52	1.80	17.89	17.89	89.41
36	23.76	8.28	2.60	25.07	25.07	83.53
48	31.68	11.04	3.46	33.63	33.63	84.04

G. Methane production rates: Phase II CSTRs

Table 10.14: Methane production rates; CSTRs Run 5

ORL (gVS/day)	Time															
	11:00:00	12:00:00	13:00:00	14:00:00	15:00:00	16:00:00	17:00:00	18:00:00	19:00:00	20:00:00	21:00:00	22:00:00	23:00:00	0:00:00	1:00:00	2:00:00
12	0.360	0.355	0.410	0.395	0.505	0.437	0.444	0.441	0.486	0.452	0.412	0.534	0.401	0.491	0.519	0.481
12	0.555	0.619	0.605	0.708	0.678	0.580	0.577	0.620	0.802	0.629	0.771	0.510	0.771	0.600	0.609	0.480
24	1.464	1.375	1.294	2.643	2.205	2.235	2.234	2.234	2.430	2.337	2.445	2.378	2.479	2.290	2.235	2.192
24	1.398	1.034	1.272	2.754	2.498	2.010	2.058	2.358	2.168	2.351	2.413	2.473	2.383	2.661	2.498	2.536
36	1.579	1.599	1.743	1.738	1.326	1.360	1.521	1.323	1.353	2.523	2.675	2.907	2.821	2.733	2.743	2.578
36	1.680	1.752	1.555	1.774	1.308	1.292	1.424	1.852	2.789	2.821	2.991	2.890	2.802	2.665	2.682	2.622
48	2.448	2.552	2.428	2.420	2.388	2.272	2.274	2.054	2.800	3.365	3.519	3.486	3.607	3.504	3.464	3.345
48	3.272	2.944	2.785	2.609	2.919	2.366	2.215	2.023	1.897	3.267	3.181	3.638	3.379	3.261	3.481	3.360

ORL (gVS/day)	Time									Mean methane rate (L CH_4/hr)	Stdev methane rate (L CH_4/hr)	∑ 12 hour period	∑24 hour period	Ratio 11:24 hr
	3:00:00	4:00:00	5:00:00	6:00:00	7:00:00	8:00:00	9:00:00	10:00:00	11:00:00					
12	0.451	0.527	0.379	0.431	0.363	0.290	0.312	0.292	0.326	0.420	0.072	5.631	10.492	0.5367147
12	0.455	0.500	0.343	0.340	0.280	0.268	0.421	0.400	0.478	0.544	0.149	8.426	13.598	0.619668
24	2.308	1.850	1.794	1.941	1.722	1.422	1.600	1.011	1.238	1.974	0.462	25.273	49.355	0.5120657
24	2.504	2.344	2.368	1.981	1.625	1.816	1.600	1.487	1.442	2.081	0.487	24.789	52.034	0.4763931
36	2.477	2.399	2.312	1.990	2.153	1.920	1.880	1.684	1.632	2.039	0.533	24.468	50.968	0.4800688
36	2.426	2.374	2.449	2.301	1.987	1.910	1.846	1.730	1.705	2.145	0.541	24.129	53.628	0.4499335
48	3.369	3.407	3.181	2.951	3.099	2.876	3.070	2.858	2.540	2.931	0.484	28.520	73.276	0.3892113
48	3.258	2.929	2.946	2.890	2.716	2.430	2.346	2.593	2.470	2.847	0.472	33.117	71.174	0.4652897

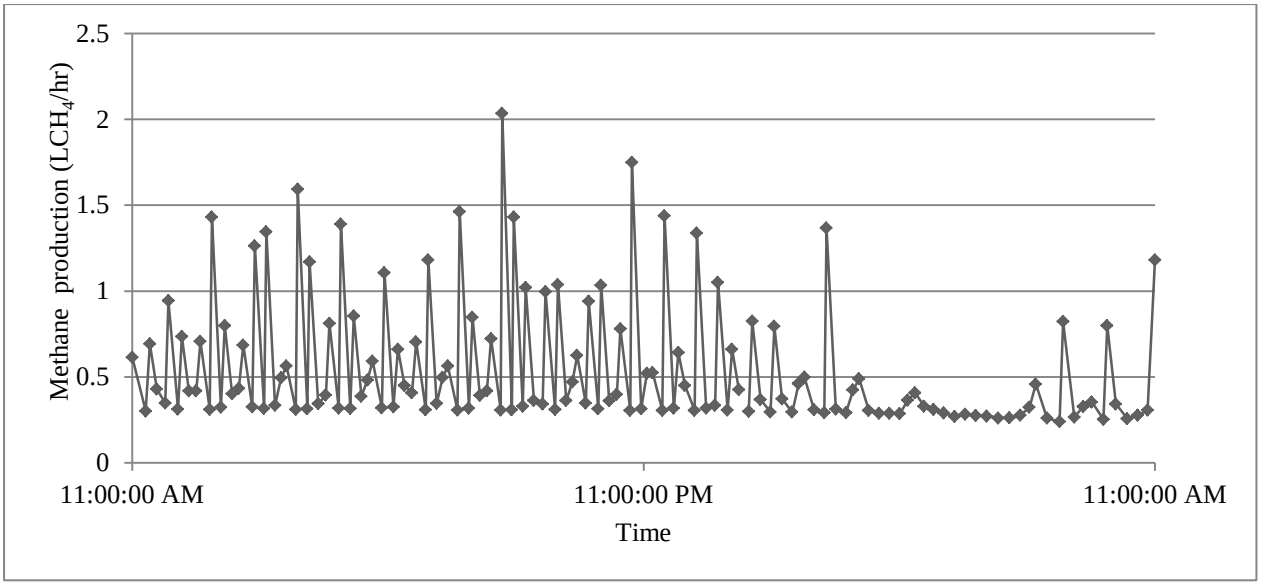

Figure 10.1: Methane production over 24 hr period at OLR 12 g VS/ day

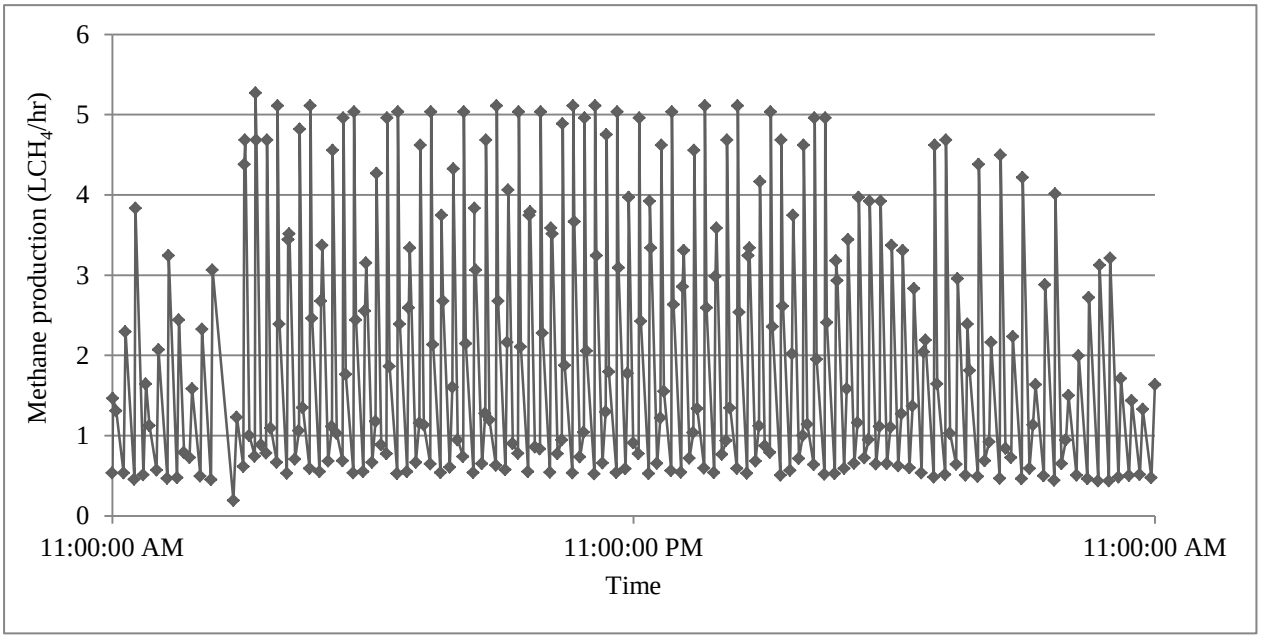

Figure 10.2: Methane production over 24 hr period at OLR 24 g VS/ day

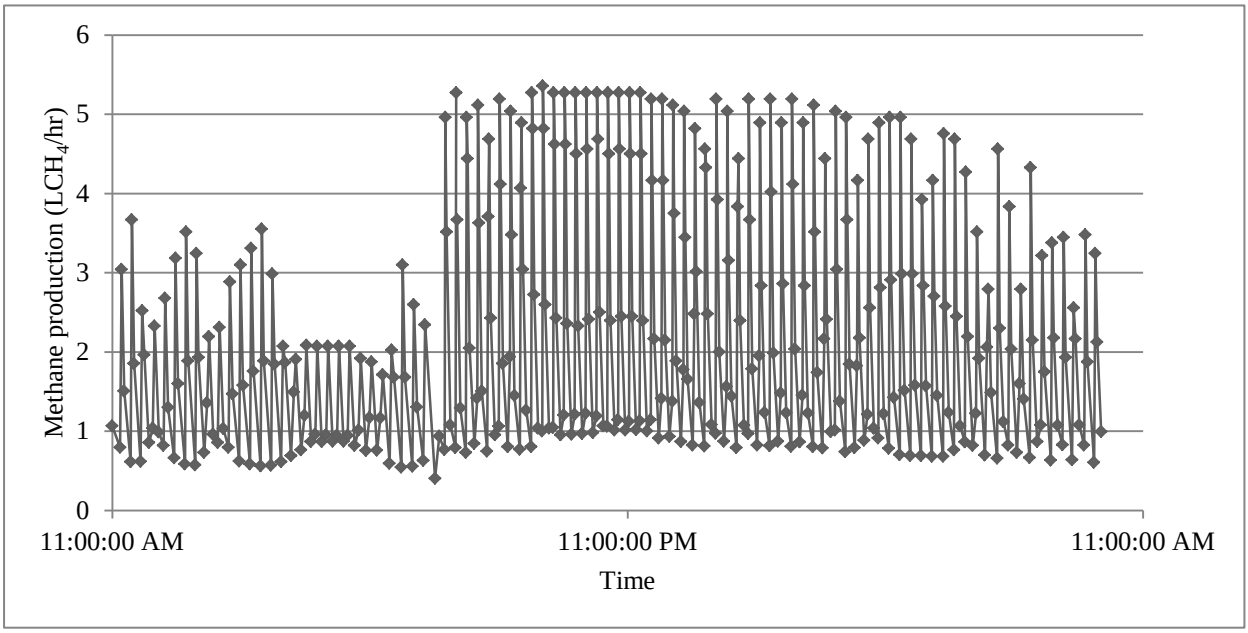

Figure 10.3: Methane production over 24 hr period at OLR 36 g VS/ day

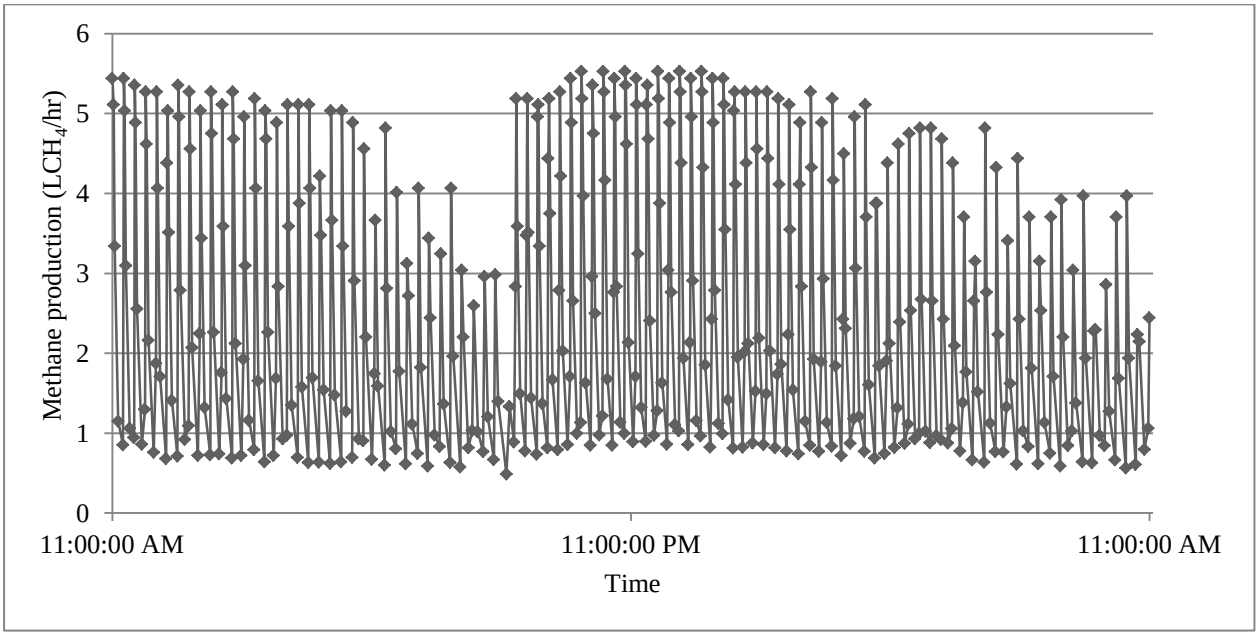

Figure 10.4: Methane production over 24 hr period at OLR 48 g VS/ day

H. Calibration curves GC for VFA concentrations

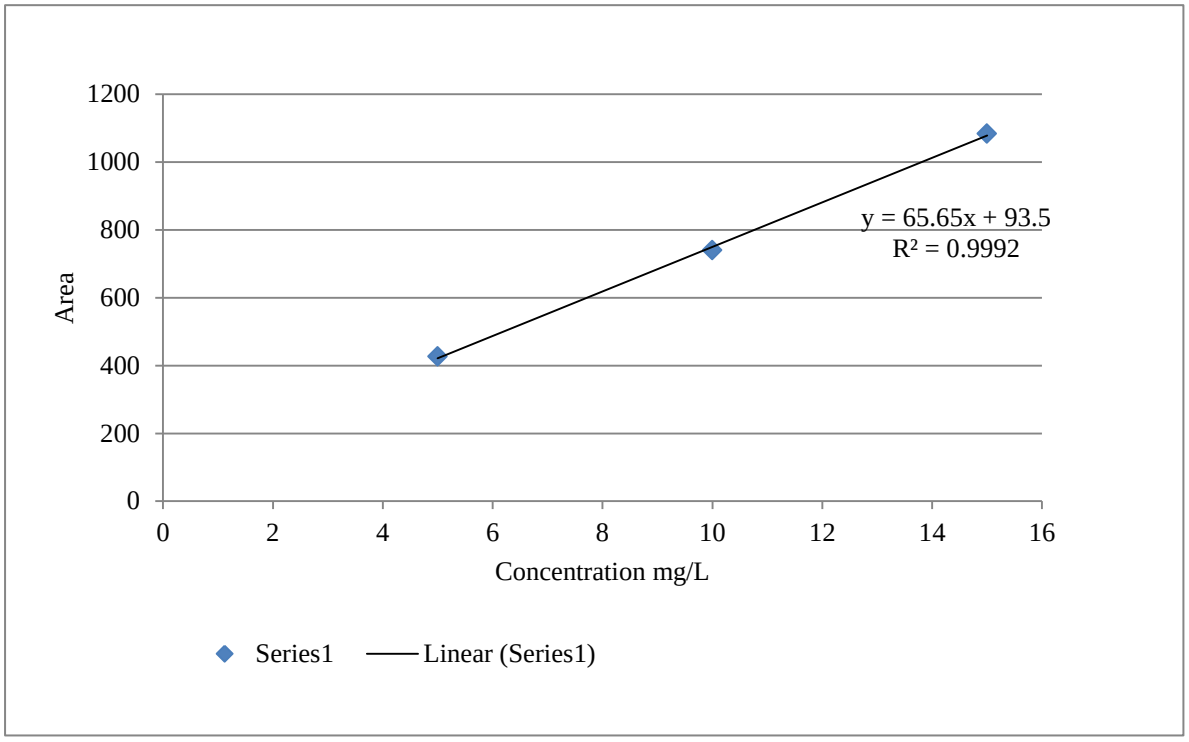

Figure 10.5: Calibration curves, acetic acid

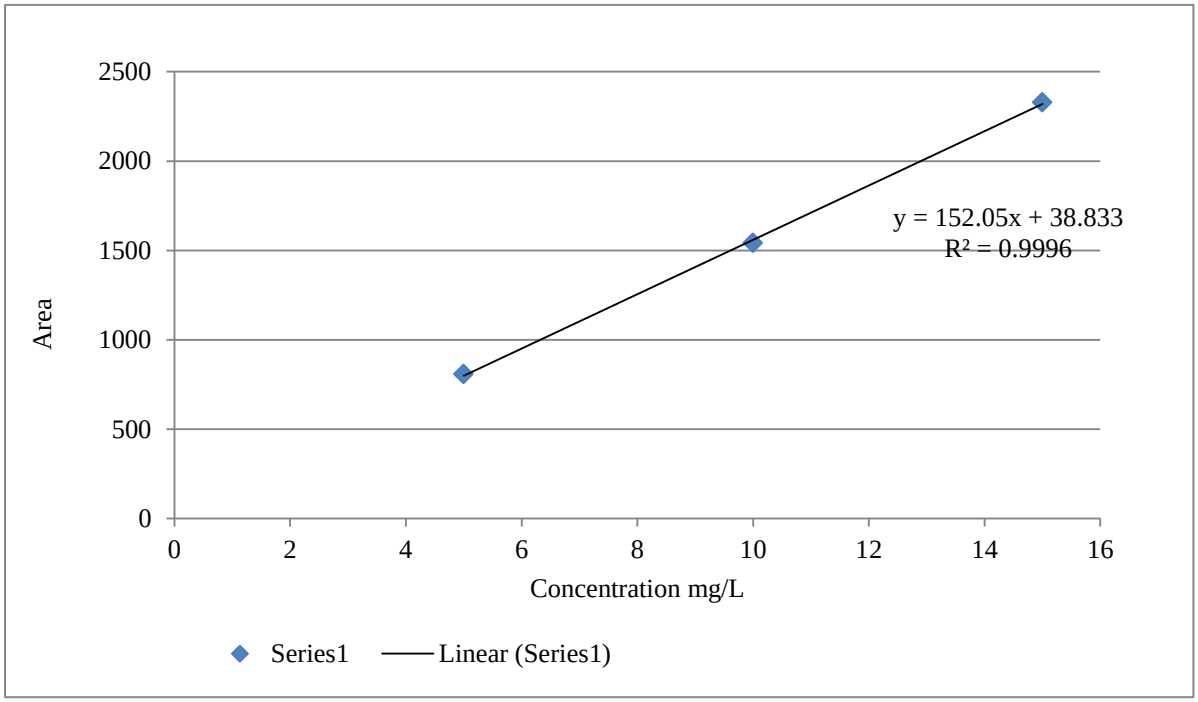

Figure 10.6:Calibration curve, butyric acid

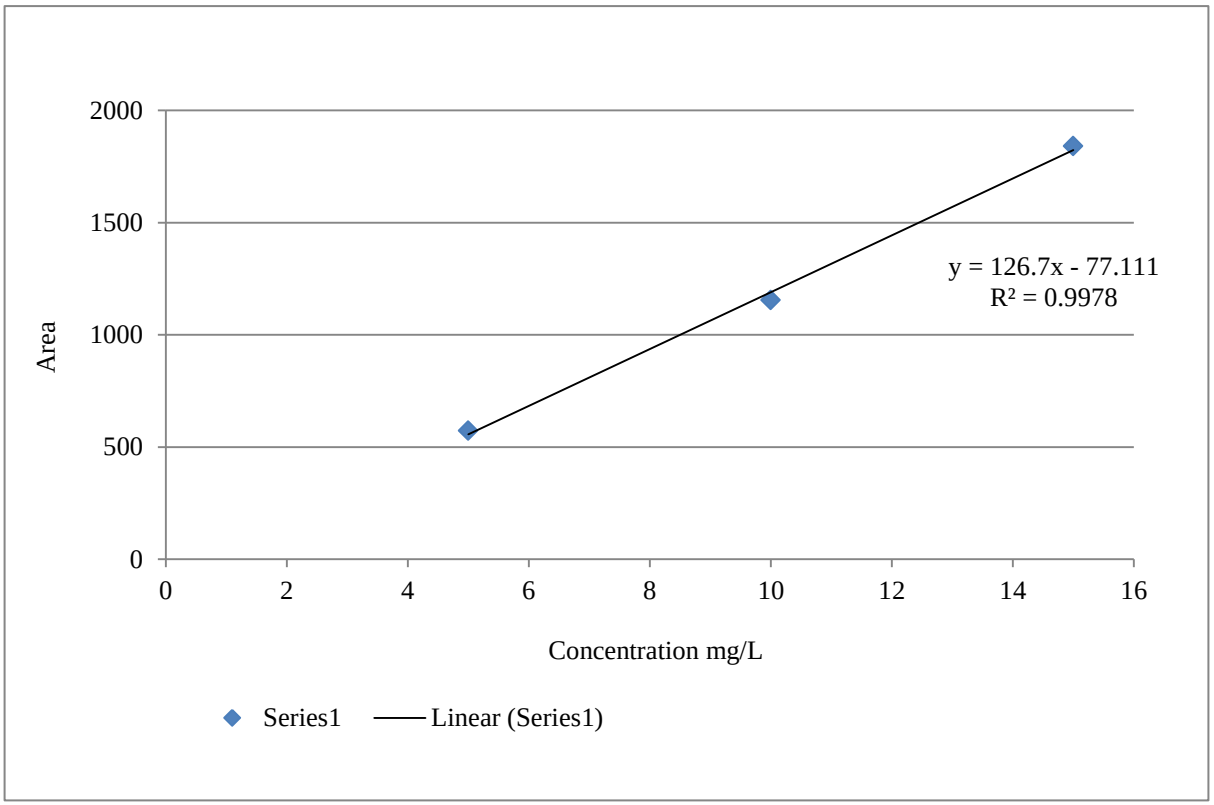

Figure 10.7: Calibration curve, propionic acid

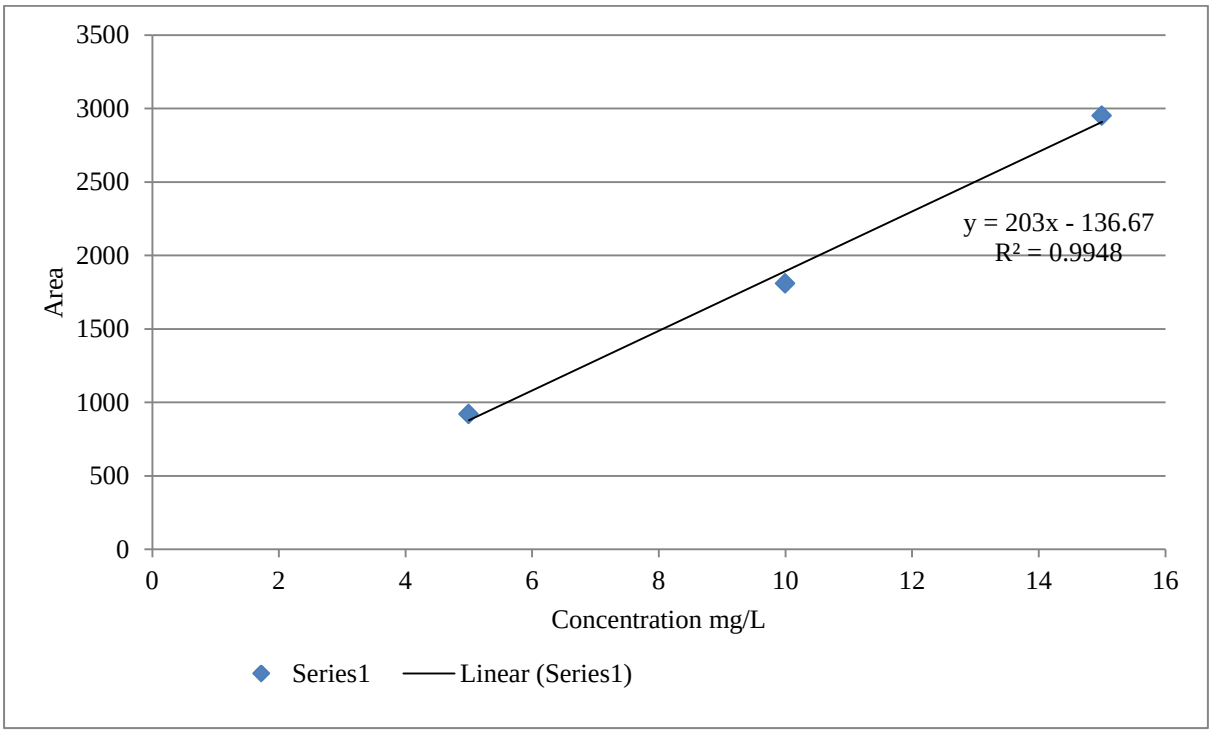

Figure 10.8: Calibration curve, isovaleric acid